EVIDENCE ON DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF FENBUTATIN OXIDE

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of reproductive toxicity are identified as the members of the Developmental and Reproductive Toxicant (DART) Identification Committee of OEHHA's Science Advisory Board (Title 22, California Code of Regulations, Section 12301) (22 CCR 12301).

Fenbutatin oxide was identified as a candidate for consideration under the "authoritative bodies" provision of Proposition 65. Subsequent to publication of a notice of intent to list this chemical, it was determined that the data used by the authoritative body did not meet the criteria specified in 22 CCR 12306(g). Pursuant to 22 CCR 12306(i), fenbutatin oxide has been referred to the DART Identification Committee. This draft document provides the DART Identification Committee with information relevant to the reproductive toxicity of this chemical. While this hazard identification document does not provide dose-response evaluation, exposure assessment or determination of allowable or safe exposure levels, the document does provide information which may be useful in such appraisals.

A public meeting of the Committee will be held on December 13, 1999, in Sacramento, California. Following discussion and Committee deliberation, the Committee will determine whether fenbutatin oxide "has been clearly shown by scientifically valid testing according to generally accepted principles" to cause reproductive toxicity.

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A. Abstract

Fenbutatin oxide is a relatively high molecular weight organotin compound. It is insoluble in water, but somewhat soluble in organic solvents. It is used in California as a selective miticide on some fruits, vegetables, select nut crops, landscaping, ornamental plants and cut flowers.

Fenbutatin oxide is poorly absorbed by the oral route (about 1% in rats). It distributes to several organs, including liver, kidney, and heart. No information on distribution to reproductive organs, placenta, or fetus was located. It has a low oral acute toxicity (LD_{50} 4400 mg/kg in rats). The chronic effect typically noted is reduced body weight.

Data on the developmental and reproductive toxicity of fenbutatin oxide come from rat and rabbit developmental studies, rat reproductive studies, a mouse dominant lethal study, and various acute, subchronic, and chronic studies. No human studies were located.

In a rat developmental study, there were reductions in the percentage of animals showing evidence of pregnancy at termination at the mid and high doses compared to controls. The effect at the mid dose was statistically significant, and the effect at the high dose was marginally significant. There was a statistically significant increase in pre-implantation losses at the high dose compared to controls. However, the mean increase in losses was greater at the low dose, although it was not statistically significant by the Wilcoxon rank sum test. There was no increase at the mid dose. Thus, there was no dose-response of the mean pre-implant losses. In evaluating causality, another important issue to consider is the point in time at which treatment was initiated. In this case treatment did not start until gd six, while implantation occurs on gestation day (gd) five to six in rats. Also, in viewing the data on implantation loss in individual animals, there are several animals with reports of more implants than corpora lutea.

In a pair of Dutch rabbit developmental studies, increases in resorptions plus early fetal deaths per litter were found. The statistical significance of these effects was not addressed by the authors of the report. In both of these studies, maternal deaths (10% to 15%) occurred among most groups, including both controls and fenbutatin oxide treated animals, in an apparently random manner.

In a New Zealand White rabbit developmental study, dose-related increases in post-implantation losses were found at the mid and high doses. However, pairwise comparisons between control and treated groups were not statistically significant by the Wilcoxon rank sum test; statistical tests for trend were not reported. Maternal deaths were observed in control (2/18; 11%), mid dose (2/18, 11%) and high dose (5/23; 22%) groups, but not in the low dose or thalidomide positive control groups. Thus, maternal mortality at the high dose exceeded the level in the controls by 11%. While the difference is not statistically significant ($p=0.3$), its biological significance is unclear.

In a three-generation rat reproduction study with two litters per generation, a small but consistent reduction in litter size was observed. This reduction was statistically significant for one litter out of six (the F1b litter). A later two-generation study found no effect on litter size. Both studies achieved comparable levels of parental toxicity (i.e. reduced body weight). However, comparison is complicated by the fact that the two

studies were conducted in different strains of rats. The later study used a larger numbers of animals, and was reported in much greater detail.

In the three-generation rat study, reduced pup survival during lactation was found in the third generation only. No effect on pup survival was found in the later two-generation study. Both studies found reduced pup body weight during lactation. Parental weights were also reduced in both studies.

In a dominant lethal study in mice, no evidence for dominant lethal or other reproductive effects was found. However, it is not clear if a systemically toxic dose was used.

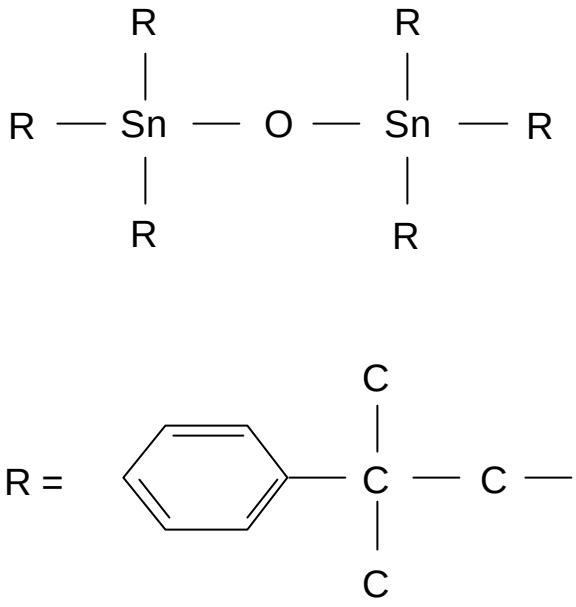
In a one-month study in rats, absolute and relative testes weights were increased. In a two-year study in rats, absolute and relative testes weights were increased at term. However, there was no effect at three, six, or twelve months in the two year study, and less than half of the animals survived to term. In the three-generation study, reduced relative testes weight was found in the F3b weanlings. In the two-generation study, the mature F1 animals had reduced absolute testes weight, but increased relative testes weight. A two-year study in dogs found no effect on absolute testes weights, but did report a significant effect on relative testes weight at the highest dose tested. Gross and/or histopathological examinations have consistently found no adverse effects on testes or ovaries attributable to fenbutatin oxide treatment in rats, mice, or dogs, however.

B. INTRODUCTION

B.1 Chemical Structure and Physical Properties

Fenbutatin oxide (Vendex; di[tri{2,2-dimethyl-2-phenylethyl}tin]oxide; SD 14114) (CAS No. 13356-08-6) is an organotin compound. The molecular weight is 1052.7. The formula is $Sn_2OC_{60}H_{78}$. The structure is shown in figure B.1.1. It is a white, crystalline solid with a melting point of approximately 145 degrees C. It is insoluble but readily dispersed in water. It is somewhat soluble in aromatic solvents (e.g. 14% w/v in benzene) (Sine 1992).

Figure B.1.1. Structure of fenbutatin oxide



B.2 Regulatory History

Fenbutatin oxide was formally identified by the U.S. Environmental Protection Agency (U.S. EPA) as causing reproductive toxicity in the course of implementation of the Toxic Release Inventory (TRI) program (*i.e.*, Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986). Fenbutatin oxide was considered for listing under Proposition 65 because of this formal identification by an authoritative body. Subsequent to publication of a notice of intent to list the chemical, it was determined that the relevant scientific criteria specified in Title 22, California Code of Regulations, Section 12306(g) (22 CCR 12306(g)) had not been met. Accordingly, as required by 22 CCR 12306(i), the chemical has been referred to the State's Qualified Experts so that they can render an opinion as to whether the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity.

B.3 California Use and Exposure Information

Fenbutatin oxide is actively registered for use in California (CDPR 1999). It is used as a selective miticide on some fruits and vegetables, select nut crops, landscaping, ornamental plants and cut flowers (Sine 1992, CDPR 1995). In California in 1995, use of 80,156 pounds was reported (CDPR 1995). The main exposures are likely to be to agricultural workers, with some exposure to the general population from consumption of fruits and vegetables, and possibly through contact with landscaping, ornamental plants, or cut flowers.

B.4 Pharmacokinetics

OEHHA staff have not been able to retrieve original reports on the pharmacokinetics of fenbutatin oxide. Information in this section is derived from secondary sources (Shell 1980a, US EPA 1994c). In general, orally administered fenbutatin oxide is poorly absorbed in rats and cows (approximately 1%). Most fenbutatin oxide was excreted rapidly, with little metabolism, in feces. Absorbed fenbutatin oxide was found in several organs and tissues, with highest concentrations in liver, kidney, and heart. Tissue levels declined rapidly after administration ceased.

In one set of experiments, groups of two male and two female rats were fed diets containing 19.5 ppm of ^{119}Sn-labeled fenbutatin oxide for one, three, or six days, or for six days followed by three or six days without fenbutatin oxide. Radioactive material was rapidly excreted in feces. During the six-day feeding period and the first day after cessation of treatment, between 93% and 99% of the administered label was recovered in feces. Less than 0.02% was recovered in urine. All tissues examined (including liver, kidney, muscle, carcass, brain and bone) contained less than 0.1 ppm of ^{119}Sn equivalents of fenbutatin oxide. The concentration of fenbutatin oxide dropped rapidly after administration ceased. Thin layer chromatography of acetone extracts of feces found that about 3.5% of the fenbutatin oxide had degraded to closely related compounds (Shell 1980a).

In another study, ^{119}Sn-labeled fenbutatin oxide was administered to Sprague-Dawley rats for one day at 10 mg/kg or 500 mg/kg, or unlabeled fenbutatin oxide was administered for 14 days at 10 mg/kg/d, followed by one day of labeled fenbutatin oxide at 10 mg/kg. Over a five-day period, 83-100% of the radioactivity was excreted in the feces and urine. Most of the fenbutatin oxide excreted in feces was unchanged, with metabolites comprising 1-3% of radioactivity. Of the tissues examined, the liver, kidney, and heart had the highest radioactivity levels. The review authors concluded that approximately 1% of the administered radioactivity was absorbed from the gastrointestinal tract, based upon the amount of radioactivity recovered in feces and cage wash (US EPA 1994c).

In a study in lactating Guernsey cows, three animals were fed ^{119}Sn-labeled fenbutatin oxide at 34 ppm in food for 21 days. Two untreated cows served as controls. The cows were sacrificed 12 hours after the end of treatment. Small amounts of radioactivity were found in the liver and kidney, ranging from 0.15 to 0.41 ppm. Trace amounts were found in the lung of one cow (0.08 ppm) and the gastrocnemius muscle of another cow (0.04 ppm). No radioactivity was found in milk, urine, brain tissue, mesenteric or subcutaneous fat, bone marrow, or quadriceps muscle. Practically all of the radioactivity was accounted for in the feces or the unabsorbed contents of the digestive tract. No metabolites of fenbutatin oxide were detected in extracts of the feces (Shell 1980a).

B.5 Non-DART Toxicities

This section draws information on the non-DART toxicities of fenbutatin-oxide from the US EPA Reregistration Eligibility Decision (RED) for fenbutatin oxide (US EPA 1994c), and not from the primary literature.

Acute Toxicity

The reported acute oral LD_{50} was 4400 mg/kg in rats, the acute dermal LD_{50} in rabbits was greater than 2000 mg/kg, and the acute inhalation LC_{50} in rats was 0.74 mg/L. The U.S. EPA did not note the cause of death in these animals. In rabbits, fenbutatin-oxide was a severe eye irritant, but produced only mild erythemia and edema when a dose of 0.5 gram was tested on skin. In guinea pigs dermal sensitization was not observed at up to 10% fenbutatin oxide solution.

Subchronic Toxicity

Systemic toxicity was not observed in rabbits after dermal application to rabbits for three weeks at doses of up to 5 mg/kg/day. Locally, erythema and edema were observed at 0.5 mg/kg/d.

Chronic Toxicity and Carcinogenicity

In rats, dietary levels of 0, 50, 100, 300 and 600 ppm for two years were not found to be carcinogenic. The NOEL for systemic toxicity was 100 ppm and the LEL was 300 ppm. The LEL was based on decreased leukocytes in female rats and reduced body weight in both sexes at all reporting points (3, 6, 12 and 24 months). At the mid- and high-dose levels, serum alkaline phosphatase was reduced and testes weight was increased in males. There was no correlative testicular histopathology.

Similarly, tumors were also observed not to increase in mice receiving the same dietary concentrations. The NOEL and LEL were 100 and 300 ppm, respectively, and were based on body weight changes.

A chronic dog study, in which fenbutatin-oxide was administered in gelatin capsules for two years at doses of 0, 2.5, 5.0, 15, 30, and 60 mg/kg/day, did not demonstrate any toxicity other than clinical observations of vomiting and diarrhea at doses of 15 mg/kg/day or more. The NOEL was 5 mg/kg/day and the LEL 15 mg/kg/day.

C. DEVELOPMENTAL TOXICITY

C.1 Overview

Four studies in experimental animals of developmental toxicity were located, one in rat and three in rabbit (Shell 1973a, 1980b, 1981). These studies contain some indications of developmental toxicity. No studies of human exposure and developmental effects were located.

C.2 Animal developmental toxicity studies

C.2.1 Rats (Shell 1980b)

Mated Wistar rats were treated with fenbutatin oxide by gavage on gestation days (gd) 6-15 at 0, 15, 30, or 60 mg/kg/d, with n = 27/group (Shell 1980b). A positive control group was treated with aspirin at 300 mg/kg/d on gd 9 with n = 15. Animals were sacrificed on gd 20. Maternal results reported included mortality, clinical observations, body weight, number of animals pregnant, and gross pathology. Fetal results reported included number of resorptions, live and dead fetuses, body weight, sex, and external, visceral, and skeletal observations.

One dam died during treatment, from the 15 mg/kg/d group; this death was reported to be probably due to mis-dosing. Statistically significant reduced body weight gain for gestation was observed at 60 mg/kg/d, for the treatment period at 30 mg/kg/d, and for gestation days 6-9 and 9-12 at 15 mg/kg/d.

The percentage of animals showing evidence of pregnancy at termination was reduced at 30 and 60 mg/kg/d. The magnitude of the reduction was larger at 30 mg/kg/d, and statistically significant only at 30 mg/kg/d. OEHHA staff has calculated that the statistical significance of the reductions was $p = 0.025$ for 30 mg/kg/d, and $p = 0.055$ for 60 mg/kg/d, using the Fisher exact test. Thus, the reduction at 60 mg/kg/d was marginally statistically significant. A statistically significant increase in pre-implantation losses was observed at 60 mg/kg/d. No other embryonic or fetal adverse effects were found. See Table C.2.1.1.

Table C.2.1.1. Selected results of rat developmental study with fenbutatin oxide (Shell 1980b). [1]

compound		Fenbutatin oxide (gd 6-15)				Aspirin (gd 9)
dose		0	15 mg/kg/d	30 mg/kg/d	60 mg/kg/d	300 mg/kg
Maternal deaths		0	1	0	0	0
Body weights of pregnant rats (g) [2]	gd 6	255.2	251.8	252.7	251.8	255.4
	gd 9	266.2	263.0*	256.8***	251.9***	267.6
	gd 15	298.7	295.2	287.7***	273.8***	299.1
	gd 20	359.9	354.5	351.0	335.9***	355.5
	Net weight gd 20 [3]	297.2	294.7	285.6***	275.6***	298.4
Pregnant/ impregnated animals		27/27 (100%)	25/26 [4] (96%)	22/27# (81%)	23/27 (85%)	14/15 (93%)
Animals with live fetuses at term (% of pregnant)		27 (100%)	25 (100%)	22 (100%)	23 (100%)	13 (93%)
Corpora lutea/ litter [2, 5]		12.8	12.8	12.8	12.6	12.8
Total implants/ litter [2, 5]		12.3	11.4	12.4	11.6	12.5
Pre-implantation loss/ litter [2, 5, 6]		0.6	1.4	0.6	1.1+	0.3
Post-implant loss/ litter [2, 5]		0.6	0.6	0.6	0.7	0.9
Resorptions/litter [2, 5]		0.6	0.6	0.6	0.7	0.8
Fetal deaths/litter [2, 5]		0.04	0.00	0.05	0.00	0.08
Live fetuses/litter [2, 5]		11.7	10.8	11.8	10.9	11.6
Live fetal weight [2]		3.49	3.52	3.50	3.50	3.13***

(1) * Significant difference from control ($P \leq 0.05$) using two-sided Student's t test.
*** Significant difference from control ($P \leq 0.001$) using two-sided Student's t test.
# Significant difference from control ($P \leq 0.05$) using Fisher's exact 2 x 2 contingency table test.
+ Significant difference from control ($P \leq 0.05$) using two-sided Wilcoxon rank sum test.
(2) Means. The authors did not report measures of variation (e.g. standard deviation).
(3) Net weight equals body weight minus uterine weight.
(4) One dead excluded
(5) Based upon number of animals (litters) with live fetuses.
(6) "When total implantations exceed the number of Corpora lutea, the value for pre-implantation losses is taken as zero." (Authors note to table entry.)

The mean pre-implantation loss at 60 mg/kg/d was 1.1, compared to 0.6 for the controls. There was a greater mean increase at the low dose (1.4), and the mid dose was the same as controls. Thus, there was no apparent dose-response trend of the mean pre-implant losses. Since the distribution of pre-implantation losses was markedly non-normal, a non-parametric test for statistical significance was used: the Wilcoxon rank sum test. At the high dose, a smaller number of pre-implantation losses were distributed among a larger number of animals than in the low dose group (see table C.2.1.2), resulting in significance in the rank sum test. The biological significance of this is not clear. Additionally, examination of individual animal data by OEHHA staff found that several animals had "negative" pre-implantation losses, i.e. more implants than corpora lutea. The authors alluded to this in a footnote to the table entry for pre-implantation losses: "When total implantations exceed the number of Corpora lutea, the value for pre-implantation losses is taken as zero."

Table C.2.1.2. Rat developmental study with fenbutatin oxide: ranked pre-implantation losses (Shell 1980b). [1]

Compound	Fenbutatin oxide (gd 6-15)				Aspirin (gd 9)
dose	0	15 mg/kg/d	30 mg/kg/d	60 mg/kg/d	300 mg/kg
Pregnant/ impregnated animals	27/27	25/26 [2]	22/27#	23/27 [3]	14/15 [4]
pre-implantation losses (corpora lutea - total implants on gd 20) (ordered)	-3	0	-2	-2	-1
	0	0	-2	-1	0
	0	0	-1	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	0	0
	0	0	0	1	2
	0	0	0	1	2
	0	0	0	1	
	0	0	0	1	
	0	0	0	1	
	0	0	0	1	
	0	1	0	2	
	0	1	1	2	
	0	1	2	2	
	0	3	4	2	
	0	4	6	11	
	1	5			
	1	8			
	2	12			
	4				
	8				

(1)# Significant difference from control ($P \leq 0.05$) using Fisher's exact 2 x 2 contingency table test.
(2) Excludes one animal that died, reported as mis-dosing accident by authors.
(3) Data for one animal was lost due to a hole punch on the paper copy.
(4) Authors did not report data for one animal except "no live fetuses."

C.2.2 Rabbits

C.2.2.1 Dutch Rabbits (Shell, 1973a)

This report (Shell 1973a) consists of 2 similar studies (Study A and Study B) in Dutch rabbits. Study B was performed due to difficulties in interpreting the results of Study A. Specifically, Study A found a small number of litters and high percentage of skeletal abnormalities at the low, but not the high, dose. In both studies, male banded Dutch rabbits were mated with female albino Dutch rabbits. The females were treated with 0, 3, or 10 mg/kg/d fenbutatin oxide or 37.5 mg/kg/d thalidomide as a positive control by oral capsule on gd 6-18. In Study A, the females were sacrificed on gestation day 28; in Study B, the females were sacrificed on gd 29. Maternal results reported included mortality, body weight and pregnancy status. Fetal results reported included live fetuses, dead fetuses, resorptions, body weight, crown to rump length, and gross, visceral, and skeletal observations.

In Study A, there were 26 control and 15 treated animals/group. Death occurred in 2 control females, 2 females treated with fenbutatin oxide at 3 mg/kg/d, and 1 female treated with fenbutatin oxide at 10 mg/kg/d. Maternal weight at day 28 was reduced (statistically significant) at 3 mg/kg/d fenbutatin oxide, but not at 10 mg/kg/d. No other information on maternal effects was provided in this report.

In Study A, in fenbutatin oxide treated animals, no effects on pregnancies, litter size, late fetal deaths or live fetal weight were observed. There was an increase in the number of resorptions plus early fetal deaths per litter at 3 mg/kg/d, but not at 10 mg/kg/d. There was an increase in the frequency of major abnormalities at 3 mg/kg/d (6.8% compared to 0% in controls), but the frequency was lower (1.5%) at 10 mg/kg/d. The authors did not report the statistical significance, if any, of these two endpoints. The authors concluded that the result in the 3 mg/kg/d group was not fenbutatin oxide treatment related, since there was no dose-related pattern. No increase in minor abnormalities was observed. No adverse effects on live litter size, late fetal deaths, fetal weight, or fetal length were observed (see Table C.2.2.1.1). In thalidomide treated animals, increases in the percentages of fetuses with major and minor abnormalities and a statistically significant reduction in live fetal weight was observed (see Table C.2.2.1.1).

In study B, there were 30 control and 20 treated animals/group. Death occurred in 2 control females, 3 females treated with fenbutatin oxide at 3 mg/kg/d, 2 females treated with fenbutatin oxide at 10 mg/kg/d, and 1 female treated with thalidomide at 37.5 mg/kg/d. Maternal weights of animals treated with fenbutatin oxide were not different from controls. Maternal weight of animals treated with thalidomide were reduced (not statistically significant) on gestation day 18, and reduced (statistically significant) on gestation day 28. No other information on maternal effects was provided in this report.

Table C.2.2.1.1. Selected results of Dutch rabbit developmental Study A with fenbutatin oxide (Shell 1973a). (1)

compound		Fenbutatin oxide (gd 6-18)			Thalidomide (gd 6-18)
dose		0	3 mg/kg/d	10 mg/kg/d	37.5 mg/kg/d
Animals mated		26	15	15	15
Maternal deaths		2	2	1	0
Number of does that aborted		0	1	0	0
Number of pregnant does surviving to term		18	9	12	11
Body weights of pregnant rabbits (g) (2)	gd 0	3890 ± 140.1	3852 ± 198.2	3634 ± 171.6	3811 ± 179.3
	gd 6	3871 ± 20.0	3829 ± 28.3	3883 ± 24.5	3911 ± 25.6
	gd 18	4045 ± 45.0	3925 ± 63.6	3945 ± 55.1	3957 ± 57.6
	gd 28	4148 ± 51.2	3940 ± 72.3*	4074 ± 62.6	4069 ± 65.4
Live litter size		7 ± 0.66	8 ± 0.93	8 ± 0.81	8 ± 0.84
Number of resorptions plus early fetal deaths/litter		0.6	1.9	0.3	1.1
Late fetal deaths/litter		0.6	0.2	0	0.1
Live fetal weight (g) (2)		35.5 ± 0.93	33.4 ± 1.31	34.2 ± 1.13	32.2 ± 1.18*
Live fetal length (cm) (2)		8.9 ± 0.13	8.4 ± 0.18	8.5 ± 0.16	8.5 ± 0.17
Number of fetuses examined for abnormalities		92	44	67	67
Fetuses with major abnormalities (%)		0	6.8	1.5	6.0
Fetuses with minor abnormalities (%)		5.4	4.5	4.5	13.4
Number of fetuses examined for unossified sternebrae or extra ribs		92	44	67	65
Fetuses with unossified 5th sternebrae (%)		25	9##	18	20
Fetuses with extra ribs (%)		23	39	30	31

(1) * $P < 0.05$ significant difference from controls using Student's t test.
$P < 0.01$ significant difference from controls Fisher's exact 2 x 2 contingency table.
(2) Mean ± SE

Table C.2.2.1.2. Selected results of Dutch rabbit developmental study B with fenbutatin oxide (Shell 1973). (1)

compound		Fenbutatin oxide (gd 6-18)			Thalidomide (gd 6-18)
dose		0	3 mg/kg/d	10 mg/kg/d	37.5 mg/kg/d
Animals mated		30	20	20	20
Maternal deaths		2	3	2	1
Number of does that aborted		0	0	2	0
Number of pregnant does surviving to term		19	16	8	14
Body weights of pregnant rabbits (g) (2)	gd 0	3826 ± 80.7	3856 ± 90.8	3648 ± 124.3	3842 ± 94.0
	gd 6	3885 ± 18.1	3912 ± 20.3	3887 ± 27.8	3890 ± 21.1
	gd 18	4042 ± 39.1	4147 ± 44.0	4054 ± 60.3	3993 ± 45.6
	gd 28	4184 ± 37.5	4237 ± 42.2	4243 ± 57.7	4041 ± 43.6
Live litter size		10 ± 0.7	8 ± 0.8	9 ± 1.1	6 ± 0.8**
Number of resorptions plus early fetal deaths/litter		0.6	1.1	1.5	1.5
Late fetal deaths/litter		0.1	0	0	0
Live fetal weight (g) (2)		34.4 ± 1.10	37.8 ± 1.20*	37.0 ± 1.70	36.2 ± 1.28
Live fetal length (cm) (2)		8.2 ± 0.09	8.5 ± 0.10	8.4 ± 0.15	8.1 ± 0.11
Number of fetuses examined for abnormalities		125	86	50	67
Fetuses with major abnormalities (%)		0.8	1.2	0	20.9
Fetuses with minor abnormalities (%)		1.6	2.3	0	16.4
Number of fetuses examined for unossified sternebrae or extra ribs		124	86	50	67
Fetuses with unossified 5th sternebrae (%)		20	20	10	10
Fetuses with extra ribs (%)		23	30	24	34

(1) * $P < 0.05$ significant difference from controls using Student's t test.
** $P < 0.01$ significant difference from controls using Student's t test.
(2) Mean ± SE

C.2.2.2 New Zealand White Rabbits (Shell, 1981)

Mated New Zealand White rabbits were treated with fenbutatin oxide by oral capsule on gd 6-18 at 0, 1, 5, or 10 mg/kg/d (n = 18, 18, 18, and 23, respectively) (Shell 1981). A positive control group was treated with thalidomide at 150 mg/kg/d on gd 8 and 9 (n = 15). Animals were sacrificed on gd 29. Maternal results reported included mortality, body weight, clinical observations, pregnancy status, and some gross pathology. Fetal results reported included live and dead fetuses, resorptions, body weights, and external, visceral, and skeletal observations.

In the maternal animals, the control group had 2 deaths and mild body weight loss (not statistically significant). Among fenbutatin oxide treated animals, the low dose group (1 mg/kg/d) did not have these effects. At 5 mg/kg/d, there were 2 deaths, mild body weight loss (not statistically significant), anorexia and gastric lesions. At 10 mg/kg/d, there were 5 deaths, severe body weight loss (statistically significant), anorexia and gastric lesions (see Table C.2.2.2.1).

In fenbutatin oxide treated animals, there was a statistically significant increase in the percentage of abortions at 10 mg/kg/d. There was an increase in the percentage of animals with total resorptions or only dead fetuses at 3 and 10 mg/kg/d, although this was not statistically significant. The percentage of females with live fetuses was reduced only at 10 mg/kg/d (statistically significant) (see Table C.2.2.2.1). There was no effect on pre-implantation loss or total implants per litter. There were small increases in resorptions and fetal deaths per litter at 3 and 10 mg/kg/d. This resulted in a dose-related increase in total post-implantation loss at 3 and 10 mg/kg/d, although the effect was not statistically significant by the Wilcoxon rank sum test. Fetal weight was reduced at 10 mg/kg/d by 25% compared to controls, although this was not statistically significant (see Table C.2.2.2.2). The lack of statistical significance at 10 mg/kg/d may have been influenced by the small sample size: only 3 animals had live fetuses. No abnormalities or anomalies attributable to fenbutatin oxide treatment were observed.

Table C.2.2.2.1. Maternal and pregnancy results of New Zealand white rabbit developmental study with fenbutatin oxide (Shell 1981). (1)

compound		Fenbutatin oxide (gd 6-18)				Thalido-mide (gd 8, 9)
dose		0	1 mg/kg/d	5 mg/kg/d	10 mg/kg/d	150 mg/kg/d
Animals mated		18	18	18	23	15
Maternal deaths		2	0	2	5	0
Body weights of pregnant rabbits (g) (2)	gd 6	4267	4214	4167	4253	4162
	gd 9	4177	4184	4139	4107**	4199
	gd 18	4110	4212	4072	3819***	4165
	gd 28	4146	4215	4049	3511**	4256
	Net weight gd 28 (3)	3888	3843	3680	3302***	3909
Evidence of pregnancy (% of mated)		14 (78%)	17 (94%)	17 (94%)	20 (87%)	12 (86%)
Abortions (% of pregnant)		3 (21%)	1 (6%)	2 (12%)	12# (60%)	2 (17%)
Total resorptions or dead fetuses only (% of pregnant)		2 (14%)	1 (6%)	4 (24%)	5 (25%)	1 (8%)
Females with live fetuses on gd 29 (% of pregnant)		9 (64%)	15 (88%)	11 (65%)	3## (15%)	9 (75%)

(1) ** $p < 0.01$ significant difference from controls using Student's t.
*** $p < 0.001$ significant difference from controls using Student's t.
# $p < 0.05$ significant difference from controls using Fisher's exact test.
$p < 0.01$ significant difference from controls using Fisher's exact test.
(2) Means. The authors did not report measures of variation (e.g. standard deviation)
(3) Net weight equals body weight minus uterine weight.

In study B, in fenbutatin oxide treated animals, there was variation in the percentage of surviving does that were pregnant: 68% in controls, 94% at 3 mg/kg/d, and 44% at 10 mg/kg/d. No adverse effects on live litter size, late fetal deaths, fetal weight , or fetal length were observed. The number of resorptions plus early fetal deaths per litter increased in a dose-related manner. The authors did not report the statistical significance, if any, of this endpoint. No increases in major or minor abnormalities were observed (see Table C.2.2.1.2). In thalidomide treated animals, reduced live litter size (statistically significant) and increased frequencies of major and minor abnormalities were observed (see Table C.2.2.1.2).

Table C.2.2.2.2. Litter results of New Zealand white rabbit developmental study with fenbutatin oxide (Shell 1981).

compound	Fenbutatin oxide (gd 6-18)				Thalido-mide (gd 8, 9)
dose	0	1 mg/kg/d	5 mg/kg/d	10 mg/kg/d	150 mg/kg/d
Number of litters with live and dead fetuses	10	16	13	5	10
Corpora lutea/litter (1)	10.3	10.4	9.8	11.4	9.9
Pre-implantation loss/ litter (1)	3.2	2.6	1.5	2.6	2.8
Total implants/ litter (1)	7.2	7.8	8.2	8.8	7.2
Resorptions/litter (1)	0.9	0.9	1.2	2.2	1.6
Fetal deaths/litter (1)	0.1	0.2	1.6	1.0	0.1
Post-implantation loss/ litter (1)	1.0	1.2	2.8	3.2	1.7
Number litters with live fetuses (2)	9	15	11	3##	9
Live fetuses/litter (3)	6.9	7.1	6.4	9.3	6.1
Live fetal weight (3)	38.8	41.4	39.2	28.9	38.7

(1) Means based on litters with live or dead fetuses. The authors did not report measures of variation (e.g. standard deviation).
No statistically significant differences from controls using Wilcoxon rank sum test.
(2) ## $p < 0.01$ significant difference from controls using Fisher's exact test.
(3) Means based on litters with live fetuses only. The authors did not report measures of variation (e.g. standard deviation).
No statistically significant differences from controls using Student's t-test.

C.3 Developmental Endpoints From Reproductive Studies

There are two reproductive studies in rats which contain observations on developmental endpoints. In these studies, fenbutatin oxide was administered to both sexes, and the period of administration included times prior to conception. It is therefore sometimes difficult to distinguish some potential female or male reproductive effects from potential developmental effects. Those effects which are more likely associated with developmental toxicity are emphasized in this section. Discussions emphasizing female and male reproductive effects are presented below (Sections D and E.).

C.3.1. Three Generation Reproduction Study (Hine Laboratories 1973)

Long-Evans rats were treated with fenbutatin oxide in food at 0, 50, 100 or 300 ppm for 3 generations, with 2 litters per generation. Parental body weights were reduced at 300 ppm in all generations (statistically significant for females in two out of three generations, and for males for all generations) (see Table C.3.1.1). Litter sizes were similar between groups, although the 300 ppm group litter sizes were consistently smaller than controls. These differences were statistically significant only for the F1b litter (see Table C.3.1.2). Pup survival during lactation was significantly reduced at 300 ppm only in the F3 litters (see Table C.3.1.3, also discussion of viability and lactation indices in section D.2.1). Birth weights were not reported. Pup weights at the end of lactation were statistically significantly reduced (see Table C.3.1.4).

Table C.3.1.1. Parental weights at sacrifice from three generation rat reproductive study (Hine Laboratories 1973). (1)

group	Female weight at sacrifice (g)				Male weight at sacrifice (g)			
	Control	50 ppm	100 ppm	300 ppm	Control	50 ppm	100 ppm	300 ppm
F0	357 ± 7.7	360 ± 7.1	366 ± 6.6	328 ± 6.9*	556 ± 14.9	542 ± 16.4	571 ± 8.4	478 ± 14.7**
F1b	361 ± 8.7	381 ± 6.8	384 ± 7.5	334 ± 5.3*	514 ± 24.6	544 ± 16.4	514 ± 17.4	442 ± 13.1*
F2b	338 ± 9.7	362 ± 6.2	352 ± 5.8	316 ± 7.9	524 ± 26.5	529 ± 12.2	541 ± 15.4	469 ± 11.0*

(1) Mean ± SE

* Significant for P = 0.05 by Dunnett's test.

** Significant for P = 0.01 by Dunnett's test.

Table C.3.1.2. Litter size from three generation rat reproductive study (Hine Laboratories 1973). (1)

group	Control	50 ppm	100 ppm	300 ppm
F1a	11.0 ± 0.56	10.8 ± 0.47	10.8 ± 0.41	9.7 ± 0.47
F1b	12.3 ± 0.45	11.4 ± 0.69	11.5 ± 0.45	9.7 ± 0.58**
F2a	10.9 ± 0.31	11.6 ± 0.32	11.5 ± 0.39	10.0 ± 0.47
F2b	11.6 ± 0.54	11.6 ± 0.63	10.6 ± 0.63	10.8 ± 0.37
F3a	10.0 ± 0.41	10.5 ± 0.38	10.1 ± 0.39	9.1 ± 0.29
F3b	11.8 ± 0.34	11.8 ± 0.42	11.8 ± 0.41	10.5 ± 0.50

(1) Mean ± SE

** Significant for p = 0.01 by Dunnett's test.

Table C.3.1.3. Litter survival during lactation from three generation rat reproductive study (Hine Laboratories 1973). (1)

group	Control	50 ppm	100 ppm	300 ppm
F1a	76.0 ± 6.13	62.9 ± 7.12	55.7 ± 8.23	52.4 ± 9.96
F1b	57.9 ± 9.00	64.7 ± 9.77	70.7 ± 7.82	57.0 ± 8.42
F2a	94.8 ± 2.00	86.0 ± 6.74	82.0 ± 7.02	92.0 ± 3.74
F2b	96.1 ± 2.32	83.5 ± 6.94	91.2 ± 5.28	94.2 ± 2.33
F3a	90.4 ± 5.40	82.3 ± 6.31	86.4 ± 5.63	62.8 ± 8.76*
F3b	90.2 ± 5.08	82.5 ± 6.72	80.4 ± 6.24	61.1 ± 8.81*

(1) Survival from pnd 1-21. Litters culled to 10 on pnd 5.
Mean percentage ± SE

* Significant for p = 0.05 by Dunnett's test.

Table C.3.1.4. Weanling body weight from three-generation rat reproductive study (Hine Laboratories 1973). (1)

Group		Control	50 ppm	100 ppm	300 ppm
F1a	Number of litters	19	17	15	12
	Weanling body weight (g)	38.2 ± 1.14	38.3 ± 0.90	37.0 ± 1.01	33.5 ± 1.63*
F1b	Number of litters	15	15	18	17
	Weanling body weight (g)	35.0 ± 1.18	38.4 ± 1.29	37.1 ± 1.12	(2)
F2a	Number of litters	18	18	18	20
	Weanling body weight (g)	34.0 ± 1.0	31.8 ± 0.96	29.8 ± 1.26*	29.0 ± 0.92**
F2b	Number of litters	18	18	18	19
	Weanling body weight (g)	35.9 ± 0.97	35.0 ± 0.71	37.1 ± 1.33	31.3 ± 0.59*
F3a	Number of litters	19	19	18	15
	Weanling body weight (g)	33.8 ± 0.69	35.3 ± 1.12	33.6 ± 1.14	29.6 ± 0.70*
F3b	Number of litters	19	18	18	15
	Weanling body weight (g)	33.4 ± 1.05	31.2 ± 0.59	32.4 ± 0.58	29.3 ± 1.46*

(1) Mean ± SE

* Significant for p = 0.05 by Dunnett's test.

** Significant for p = 0.01 by Dunnett's test.

(2) Data lost in copying.

C.3.2. Two Generation Reproduction Study (du Pont 1990)

In the second study (du Pont 1990), Crl:CDrBR rats were treated with fenbutatin oxide in food at 0, 40, 75, 250, or 500 ppm for 2 generations, with 1 litter per generation. Mean daily intake of fenbutatin oxide during gestation days 0-14 for the first/second generations were 0/0, 2.72/2.86, 5.10/5.36, 16.3/18.0, and 32.1/41.6 mg/kg/d. Parental (male and female) body weights were consistently and statistically significantly reduced at 500 ppm for premating, gestation, and lactation periods in both generations (see Tables C.3.2.1 and C.3.2.2). Additionally, body weight gain was also statistically significantly reduced in the P1 females during the premating period at 250 ppm. Parental food consumption was reduced at 500 ppm and among P1 females at 250 ppm. No effects on litter size or birth weight were observed (see Table C.3.2.3). Postnatal survival was unaffected (see Table C.3.2.4). At 500 ppm, reduced pup body weight was observed during lactation (see Table C.3.2.5).

Table C.3.2.1. Parental male weights in two generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1 males	Day 0	308.6 ± 30.4	309.5 ± 28.8	312.6 ± 31.0	310.1 ± 29.8	305.9 ± 27.8
	Day 70 (2)	575.3 ± 40.0	585.4 ± 47.1	582.4 ± 55.4	573.5 ± 55.6	484.3 ± 55.8*
	Day 119	636.1 ± 53.4	661.8 ± 58.2	649.5 ± 68.1	633.6 ± 68.5	523.8 66.7*
F1 males	Day 0	59.8 ± 5.4	62.6 ± 7.3	65.6 ± 6.1	58.5 ± 7.4	44.8 ± 6.1*
	Day 105 (2)	592.1 ± 59.9	598.4 ± 64.5	607.6 ± 56.4	581.5 ± 67.2	454.2 ± 47.8*
	Day 154	650.2 ± 64.4	655.9 ± 77.1	670.4 ± 66.3	640.1 ± 73.8	494.8 ± 54.9*

(1) All values in grams: mean ± SD

* Statistically significant by One-way Analysis of Variance and Dunnett's test at alpha = 0.05.

(2) End of premating phase.

Table C.3.2.2. Parental female weights in two generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1 females	Premating day 0	227.1 ± 17.8	229.7 ± 19.9	228.0 ± 21.5	225.3 ± 15.7	226.0 ± 18.6
	Premating day 70 (2)	313.8 ± 23.1	317.5 ± 28.7	317.8 ± 34.5	300.1 ± 19.5	277.1 ± 23.2*
	Gestation day 0	309.8 ± 20.0	315.0 ± 25.3	317.2 ± 38.3	301.7 ± 20.3	271.3 ± 21.7*
	Gestation day 21	472.4 ± 36.7	466.1 ± 30.9	467.4 ± 45.3	452.8 ± 44.0	407.2 ± 31.9*
	Lactation day 1	354.5 ± 27.0	359.3 ± 22.8	361.8 ± 33.9	342.4 ± 27.7	301.1 ± 22.9*
	Lactation day 21	355.6 ± 23.4	364.0 ± 22.2	368.6 ± 27.1	363.1 ± 30.3	334.7 ± 19.1*
F1 females	Premating day 0	56.7 ± 5.2	58.1 ± 6.0	58.5 ± 5.9	53.8 ± 7.5	42.4 ± 4.7*
	Premating day 105 (2)	323.5 ± 34.1	329.1 ± 47.9	335.2 ± 43.4	315.3 ± 32.8	256.7 ± 23.9*
	Gestation day 0	321.3 ± 34.9	322.4 ± 33.7	336.0 ± 39.2	313.6 ± 30.2	255.6 ± 24.3*
	Gestation day 21	477.5 ± 41.2	471.0 ± 43.6	486.5 ± 49.1	460.9 ± 39.5	388.6 ± 36.4*
	Lactation day 1	373.1 ± 34.4	371.7 ± 45.5	373.0 ± 39.3	356.6 ± 25.4	291.2 ± 24.1*
	Lactation day 21	352.8 ± 30.6	365.7 ± 37.1	362.4 ± 28.4	347.0 ± 32.1	292.9 ± 32.3*

(1) All values in grams: mean ± SD

* Statistically significant by One-way Analysis of Variance and Dunnett's test at alpha = 0.05

(2) End of premating phase.

Table C.3.2.3. Litter data from two generation reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
F1 generation	Litter size	14.5 ± 3.58	14.0 ± 3.74	12.8 ± 4.26	13.0 ± 5.96	13.1 ± 3.17
	Live litter size	14.2 ± 3.56	13.8 ± 3.67	12.6 ± 4.08	12.5 ± 6.06	13.0 ± 3.16
	Birth weight (g)	6.8 ± 0.63	7.0 ± 0.63	7.0 ± 0.76	6.8 ± 0.84	7.0 ± 0.96
F2 generation	Litter size	12.3 ± 4.66	13.5 ± 3.81	14.5 ± 1.65	13.3 ± 3.59	12.4 ± 2.89
	Live litter size	12.1 ± 4.49	13.2 ± 3.82	13.8 ± 3.13	13.3 ± 3.57	12.3 ± 2.86
	Birth weight (g)	6.8 ± 0.72	7.0 ± 1.03	6.9 ± 0.86	6.6 ± 0.56	6.7 ± 0.46

(1) Data are mean ± S.D.
No statistically significant effects were found using the Mann-Whitney U test on litter means at alpha = 0.05.

Table C.3.2.4. Viability and lactation indices from two generation rat reproduction study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
F1 generation	Viability index (2)	98.4 ± 3.19	99.2 ± 2.22	98.7 ± 2.59	97.8 ± 6.19	98.9 ± 3.50
	Lactation index (3)	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
F2 generation	Viability index (2)	99.4 ± 2.67	97.2 ± 7.32	98.7 ± 3.70	94.5 ± 18.65	98.3 ± 4.78
	Lactation index (3)	99.4 ± 2.67	99.3 ± 3.03	100.0 ± 0.00	95.3 ± 15.99	100.0 ± 0.00

(1) Mean ± S.D.
No statistically significant differences were found using the Mann-Whitney U test on litter means at alpha = 0.05
(2) Viability index = (number alive on postnatal day 4/number alive on postnatal day 0) x 100
(3) Lactation index = (number alive on postnatal day 21/number alive on postnatal day 4 [postcull]) x 100

Table C.3.2.5. Pups weights during lactation from two generation rat reproduction study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
F1 generation	Postnatal day 4 (precull)	11.0 ± 1.54	11.5 ± 1.89	12.0 ± 1.93	10.9 ± 2.52	10.3 ± 1.72
	Postnatal day 4 (postcull)	11.1 ± 1.41	11.4 ± 1.92	12.0 ± 1.97	10.9 ± 2.55	10.3 ± 1.75*
	Postnatal day 21	58.9 ± 5.28	61.4 ± 5.49	63.1 ± 4.89	57.7 ± 7.60	44.7 ± 4.76*
F2 generation	Postnatal day 4 (precull)	11.6 ± 2.26	11.2 ± 2.71	11.4 ± 1.17	10.5 ± 2.32	10.1 ± 1.35*
	Postnatal day 4 (postcull)	11.7 ± 2.15	11.4 ± 2.75	11.3 ± 1.08	10.4 ± 2.35	10.1 ± 1.40*
	Postnatal day 21	59.5 ± 6.64	59.8 ± 6.42	60.1 ± 4.82	53.7 ± 6.97*	42.1 ± 5.46*

(1) Data in grams: mean ± S.D

* Significantly different from control using Mann-Whitney U test on litter means at alpha = 0.05

C.4 Other relevant information

Information on the general pharmacokinetics of fenbutatin oxide is presented in section B.4. No information was located on the placental transfer or fetal accumulation of fenbutatin oxide. When Guernsey cows were treated with 34 ppm fenbutatin oxide in food for 21 days, no fenbutatin oxide was reported in the milk. The limit of detection was not specified for this result (Shell 1980a).

C. 5 Integrative evaluation.

The data on the possible developmental toxicity of fenbutatin oxide come primarily from one rat and three rabbit developmental studies (Shell 1973a, 1980b, 1981), supplemented with results from two multigeneration studies in rats.

In the rat developmental study (Shell 1980b), there were reductions in the percentage of animals with evidence of pregnancy at termination at the mid and high doses (30 and 60 mg/kg/d). This was statistically significant ($p = 0.025$) at the mid dose, and marginally significant at the high dose ($p = 0.055$). There was a statistically significant increase in pre-implantation losses at the high dose compared to controls (Wilcoxon rank sum test);however, in terms of mean loss, differences between control and treated animals were small, with mean loss greater at the low dose than the high dose and the mid dose not differing from controls. Thus, mean losses showed no clear dose-response trend. Additionally, there were several animals where the number of implants exceeded the number of corpora lutea.

The statistically significant increase in pre-implantation losses was evidently one of the effects referred to in the citation supporting addition of fenbutatin oxide to the TRI list (US EPA 1994a).

In this study, exposure to fenbutatin oxide began on gestation day six. In rats, implantation is generally regarded as occurring five to six days after conception (e.g. Miller 1983). The U.S. EPA guidelines for developmental toxicity risk assessment (U.S. EPA 1991) make the following statement regarding preimplantation losses:

> "If treatment begins around the time of implantation (i.e. day 6 of gestation in the mouse, rat or rabbit), an increase in preimplantation loss probably reflects variability that is not treatment-related in the animals being used, but the data should be examined carefully to determine if there is a dose-response relationship. If preimplantation loss is related to dose, further studies would be necessary to determine the mechanism and extent of such effects."

Two developmental studies were conducted in Dutch rabbits (Shell 1973a). The second study was conducted due to difficulty in interpreting the results from the first study. In the first study, Study A, there was an increase in resorptions plus early fetal deaths per litter at the low dose (3 mg/kg/d) but not the high dose (10 mg/kg/d). Statistical significance values for the observations were not reported by the authors. Maternal deaths occurred in the vehicle control and both treatment groups in a non dose-related fashion In Study B, the number of resorptions plus early fetal deaths per litter was

increased in a dose-related manner. Measures of statistical significance for this observation were not reported. As in the earlier study, maternal deaths occurred in the negative control and both treatment groups in a manner apparently unrelated to treatment.

In a New Zealand White rabbit developmental study (Shell 1981), there were dose-dependent increases in mean post-implantation losses at the mid and high doses (5 and 10 mg/kg/d). However, pairwise comparisons between control and treated groups were not statistically significant using the Wilcoxon Rank sum test; trend tests were not reported. There was a high (60%) and statistically significant percentage of abortions at the high dose. Maternal deaths were observed in control (2/18; 11%), mid dose (2/18, 11%) and high dose (5/23; 22%) groups, but not in the low dose or thalidomide positive control groups.

The increase in post-implantation losses at the mid dose in this study is one of the effects referred to in the citation supporting addition of fenbutatin oxide to the TRI list (US EPA 1994a).

The U.S. EPA developmental toxicity guidelines (US EPA 1991) make the following statement regarding maternal toxicity:

> "Agents that produce developmental toxicity at a dose that is not toxic to the maternal animal are especially of concern because the developing organism is affected but toxicity is not apparent in the adult. However, the more common situation is when adverse developmental effects are produced only at doses that cause minimal maternal toxicity: in these cases, developmental effects are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity. At doses causing excessive maternal toxicity (that is, significantly greater than the minimal toxic dose), information on developmental effects may be difficult to interpret and of limited value."

In a section on study design, minimal toxicity is described as follows:

> "The high dose is selected to produce some minimal maternal or adult toxicity (i.e. a level that at the least produced marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity, and at the most produces no more than 10% mortality)."

In the developmental study in New Zealand White rabbits (Shell 1981), maternal mortality at the high dose (22%) exceeded the level in the controls (11%) by 11%. While the difference is not statistically significant ($p=0.3$), its biological significance is unclear.

In one of two multigeneration rat studies (Hine Laboratories 1973), litter size in the high concentration (300 ppm) group was consistently smaller than controls, although this was statistically significant only for the F1b litter. Parental body weights were reduced at this high concentration. In the second study (du Pont 1990), there was no consistent or statistically significant effect on litter size at concentrations of fenbutatin oxide at up to 500 ppm. Parental body weights were also reduced at the high concentration in this study. In the rat developmental study, no effect on litter size was found (Shell 1980b). It should be noted that comparison of the developmental and the reproductive studies is complicated by the use of a different strain of rats in each study. In the earlier rat reproduction study (Hine Laboratories 1973), reduced postnatal survival was observed in both litters of the third generation. Reduced pup weight at weaning was consistently

found. In the later rat reproduction study (du Pont 1990), no effect on pup survival was found. It should be noted that this was a two-generation study, so does not directly contradict the finding of reduced pup survival in the third generation of the earlier study. Pup body weights were also reduced during lactation in the later study.

The statistically significant reduction in postnatal survival in the three-generation reproductive study was one of the effects referred to in the citation supporting addition of fenbutatin oxide to the TRI list (US EPA 1994a).

As the statute is currently interpreted, developmental effects resulting from postnatal exposure are outside the purview of Proposition 65 (OEHHA 1996). It is possible that the effects on lactating pups which were seen in the rat reproductive studies were due to adverse effects which occurred prenatally, but were manifested postnatally. It is also possible that they were due to transfer of fenbutatin oxide through the milk, or from pups eating their mother's food. Additionally, it is possible that they were due to problems with lactation in the mother. Hence, in the absence of data obtained from a study protocol involving cross-fostering of pups, the observed postnatal effects cannot be conclusively attributed to prenatal exposure to fenbutatin oxide.

D. FEMALE REPRODUCTIVE TOXICITY

D.1 Overview

Data on the female reproductive toxicity of fenbutatin oxide comes from two multigeneration studies in rats, supplemented by acute studies in rats and chronic studies in rats, mice, and dogs. No studies concerning human exposure and reproductive effects were located.

D.2 Multigeneration Studies (Rats)

In the two multigeneration studies in rats, fenbutatin oxide was administered to both sexes. It is therefore sometimes difficult to distinguish some potential female reproductive effects from potential male reproductive effects. Those effects which are more likely to be associated with female reproductive toxicity are emphasized in this section. A discussion emphasizing male reproductive effects is presented below (Section E.).

D.2.1. Three Generation Reproduction Study (Hine Laboratories 1973)

In the earlier reproduction study (Hine Laboratories 1973), Long-Evans rats were fed a diet containing 0, 50, 100 or 300 ppm fenbutatin oxide for three generations, with two litters per generation. There were 20 females and 10 males per group. The second litter of each generation became the parental group of the next generation. Litters were culled to 10 on postnatal day (pnd) 5. Parental endpoints reported included survival, body weight, clinical observations and gross pathology. Reproductive endpoints reported included fertility, litter size, postnatal survival, postnatal (but not birth) weights, and selected pup organ weights and histopathology.

No parental deaths were reported. Parental body weights at sacrifice were consistently reduced at 300 ppm (statistically significant for females in 2 out of 3 generations, and for males in all generations). The magnitude of the reduction in weight was 6-8% for females and 10-14% for males (see Table D.2.1.1). Hyperactivity and irritability were also reported at 300 ppm.

No effects on fertility indices (number pregnant/number mated on a per female basis) were observed. No effects on gestation indices (number of litters with live pups/number of pregnancies) were observed. Litter sizes were similar, although the 300 ppm group litter sizes were consistently smaller than controls. These differences were statistically significant only for the F1b litter (see Table D.2.1.2). Birth weights were not reported.

Reduced postnatal survival (statistically significant) for pnd 1-21 was found in only the F3a and F3b litters at 300 ppm (see Table D.2.1.3). Reduced postnatal survival (statistically significant) between pnd 1-5 or 5-21 was observed in several litters at all treatment levels, but the authors discount these results due to an apparently random distribution and a lack of consistent trends or patterns (see Tables D.2.1.4 and D.2.1.5).

The discrepancy between these results likely arises from different statistical treatments. The statistical significance of the pnd 1-5 and 5-21 results was tested using individual pup numbers, whereas pnd 1-21 was tested using litter-based numbers, with litters culled to 10. Reduced pup weight (statistically significant) on pnd 21 was found in 5 of 6 litters at 300 ppm. Reduced pup weight (statistically significant) on pnd 21 was also found in the F2a litter at 100 ppm (see Table D.2.1.6). For the F3b weanlings, the relative testes weight was reduced (statistically significant) at 100 and 300 ppm. No effects on relative brain, heart, liver, or kidney weights were observed (see Table D.2.1.7).

Table D.2.1.1. Parental weights at sacrifice from three generation rat reproductive study (Hine Laboratories 1973). (1)

group	Female weight at sacrifice (g)				Male weight at sacrifice (g)			
	Control	50 ppm	100 ppm	300 ppm	Control	50 ppm	100 ppm	300 ppm
F0	357 ± 7.7	360 ± 7.1	366 ± 6.6	328 ± 6.9*	556 ± 14.9	542 ± 16.4	571 ± 8.4	478 ± 14.7**
F1b	361 ± 8.7	381 ± 6.8	384 ± 7.5	334 ± 5.3*	514 ± 24.6	544 ± 16.4	514 ± 17.4	442 ± 13.1*
F2b	338 ± 9.7	362 ± 6.2	352 ± 5.8	316 ± 7.9	524 ± 26.5	529 ± 12.2	541 ± 15.4	469 ± 11.0*

(1) Mean ± SE

* Significant for P = 0.05 by Dunnett's test.

** Significant for P = 0.01 by Dunnett's test.

Table D.2.1.2. Litter size at birth from three generation rat reproductive study (Hine Laboratories 1973). (1)

group	Control	50 ppm	100 ppm	300 ppm
F1a	11.0 ± 0.56	10.8 ± 0.47	10.8 ± 0.41	9.7 ± 0.47
F1b	12.3 ± 0.45	11.4 ± 0.69	11.5 ± 0.45	9.7 ± 0.58**
F2a	10.9 ± 0.31	11.6 ± 0.32	11.5 ± 0.39	10.0 ± 0.47
F2b	11.6 ± 0.54	11.6 ± 0.63	10.6 ± 0.63	10.8 ± 0.37
F3a	10.0 ± 0.41	10.5 ± 0.38	10.1 ± 0.39	9.1 ± 0.29
F3b	11.8 ± 0.34	11.8 ± 0.42	11.8 ± 0.41	10.5 ± 0.50

(1) Mean ± SE

** Significant for p = 0.01 by Dunnett's test.

Table D.2.1.3. Litter survival during lactation from three generation rat reproductive study (Hine Laboratories 1973). (1)

group	Control	50 ppm	100 ppm	300 ppm
F1a	76.0 ± 6.13	62.9 ± 7.12	55.7 ± 8.23	52.4 ± 9.96
F1b	57.9 ± 9.00	64.7 ± 9.77	70.7 ± 7.82	57.0 ± 8.42
F2a	94.8 ± 2.00	86.0 ± 6.74	82.0 ± 7.02	92.0 ± 3.74
F2b	96.1 ± 2.32	83.5 ± 6.94	91.2 ± 5.28	94.2 ± 2.33
F3a	90.4 ± 5.40	82.3 ± 6.31	86.4 ± 5.63	62.8 ± 8.76*
F3b	90.2 ± 5.08	82.5 ± 6.72	80.4 ± 6.24	61.1 ± 8.81*

(1) Survival from pnd 1-21. Litters culled to 10 on pnd 5.
Mean percentage ± SE

* Significant for p = 0.05 by Dunnett's test.

Table D.2.1.4. Viability indices from three generation rat reproductive study (Hine Laboratories 1973). (1)

group	Control	50 ppm	100 ppm	300 ppm
F1a	92.2	81.9**	75.1**	64.4**
F1b	93.6	89.8	94.8	90.8
F2a	98.0	87.1**	93.5*	96.0
F2b	93.8	86.1**	93.1	97.1
F3a	93.5	92.4	88.0	81.4**
F3b	94.1	85.2**	83.0**	82.9**

(1) Viability index: percentage of pups surviving from pnd 1 to pnd 5.
The authors did not report indications of variation (e.g. standard deviation). The values were based on pup data per group, which does not have variability.
* Significant for p = 0.05 by Chi square for pups.
** Significant for p = 0.01 by Chi square for pups.

Table D.2.1.5. Lactation indices from three generation rat reproductive study (Hine Laboratories 1973).

group	Control	50 ppm	100 ppm	300 ppm
F1a	84.1	75.9	70.2**	75.2
F1b	61.8	68.5	73.0*	61.9
F2a	96.0	96.6	87.0**	94.0
F2b	98.8	95.7	96.4	95.6
F3a	94.9	85.2**	94.4	77.7**
F3b	94.2	91.0	90.4	70.8**

(1) Lactation index: percentage of pups surviving from pnd 5(after culling to 10 pups/litter) to pnd 21.
The authors did not report indications of variation (e.g. standard deviation). The values were based on pup data per group, which does not have variability.
* Significant for p = 0.05 by Chi square for pups
** Significant for p = 0.01 by Chi square for pups

Table D.2.1.6. Weanling body weight from three-generation rat reproductive study (Hine Laboratories 1973). (1)

Group		Control	50 ppm	100 ppm	300 ppm
F1a	Number of litters	19	17	15	12
	Weanling body weight (g)	38.2 ± 1.14	38.3 ± 0.90	37.0 ± 1.01	33.5 ± 1.63*
F1b	Number of litters	15	15	18	17
	Weanling body weight (g)	35.0 ± 1.18	38.4 ± 1.29	37.1 ± 1.12	(2)
F2a	Number of litters	18	18	18	20
	Weanling body weight (g)	34.0 ± 1.0	31.8 ± 0.96	29.8 ± 1.26*	29.0 ± 0.92**
F2b	Number of litters	18	18	18	19
	Weanling body weight (g)	35.9 ± 0.97	35.0 ± 0.71	37.1 ± 1.33	31.3 ± 0.59*
F3a	Number of litters	19	19	18	15
	Weanling body weight (g)	33.8 ± 0.69	35.3 ± 1.12	33.6 ± 1.14	29.6 ± 0.70*
F3b	Number of litters	19	18	18	15
	Weanling body weight (g)	33.4 ± 1.05	31.2 ± 0.59	32.4 ± 0.58	29.3 ± 1.46*

(1) Mean ± SE

* Significant for p = 0.05 by Dunnett's test.

** Significant for p = 0.01 by Dunnett's test.

(2) Data lost in copying.

Table D.2.1.7. Relative organ weights for F3b weanlings in three-generation reproductive study (Hine Laboratories 1973). (1)

Organ	males				females			
	0	50 ppm	100 ppm	300 ppm	0	50 ppm	100 ppm	300 ppm
Brain	3.76 ± 0.051	3.60 ± 0.054	3.71 ± 0.049	3.75 ± 0.118	3.58 ± 0.060	3.54 ± 0.050	3.56 ± 0.062	3.80 ± 0.139
Heart	0.65 ± 0.022	0.65 ± 0.033	0.67 ± 0.046	0.65 ± 0.019	0.66 ± 0.016	0.66 ± 0.020	0.68 ± 0.035	0.68 ± 0.021
Liver	4.16 ± 0.076	4.15 ± 0.158	4.04 ± 0.152	4.06 ± 0.034	4.29 ± 0.056	4.46 ± 0.017	4.25 ± 0.074	(2)
Kidney	1.29 ± 0.027	1.25 ± 0.048	1.29 ± 0.029	1.25 ± 0.026	1.31 ± 0.026	1.33 ± 0.031	1.26 ± 0.034	1.38 ± 0.046
Testes	0.38 ± 0.015	0.38 ± 0.014	0.32 ± 0.014*	0.32 ± 0.007 **	NA	NA	NA	NA
Number of animals	10	5	5	10	10	5	5	10

(1) Mean ± SE, except for numbers of animals.
Organ weights expressed as (organ weight/body weight) x 100
* Significant at P = 0.05 by Dunnett's test
**Significant at P = 0.01 by Dunnett's test
(2) Data lost in copying

D.2.2 Two Generation Reproduction Study (du Pont 1990)

In the later reproduction study, Crl:CDrBR rats were fed 0, 40, 75, 250, or 500 ppm fenbutatin oxide for 2 generations, with 1 litter per generation. There were 30 females and 30 males per group. Parental endpoints reported included survival, body weight, food consumption and clinical observations. Reproductive endpoints reported included histopathology of parental reproductive tissues, mating, gestation, and fertility indices, litter size, birth weight, postnatal survival and weight, and gross and microscopic pathology of pups.

In the P1 generation, 1 male rat in the 500 ppm group died. In the F1 generation, 2 males in the 500 ppm group, 1 male in the 250 ppm group, 1 female in the 500 ppm group, 1 female in the 75 ppm group, and 2 females in the control group died. Deaths were attributed to several unrelated causes. The authors concluded that none of the deaths were attributable to fenbutatin oxide treatment. Parental (male and female) body weights and weight gains during the premating, gestation, and lactation periods were consistently, statistically significantly reduced at 500 ppm (see Tables D.2.2.1 and D.2.2.2). Male body weights at the end of the premating phase were reduced (statistically significant) by approximately 16% (P1) and 23% (F1) at 500 ppm compared to controls. Among P1 females at 500 ppm, mean body weights were approximately 12% lower than controls on day 0 of gestation, and 14% lower on day 21 of gestation; these reductions were statistically significant. Among F1 females at 500 ppm, mean body weights were approximately 20% lower than controls on day 0 of gestation, and 19% lower on day 21 of gestation; these reductions were also statistically significant. (see Table D.2.2.2). Body weight gain was also reduced (statistically significant) in the P1 females during the premating period at 250 ppm. Parental food consumption was reduced at 500 ppm and among P1 females at 250 ppm. No adverse clinical observations or gross or microscopic pathological effects (including effects on ovaries or uterus) attributable to fenbutatin oxide treatment were reported.

Breeding was performed one male to one female. No effects on mating index (number copulating/number cohoused) were observed. No effect on the fertility index (number delivering/number copulating) was observed in the P1 generation. The fertility index was lower than controls (not statistically significant) in the F1 females at 40 and 75 ppm, but not at 250 or 500 ppm (see Table D.2.2.3). No effect on gestation length was observed. No effects on litter size or birth weight were observed (see Table D.2.2.4). No effects on pup survival were observed (see Table D.2.2.5). Pup body weights in both generations were consistently reduced (statistically significantly in most instances) during lactation at 500 ppm. Pup body weights in the F2 generation were also reduced (statistically significant) on postnatal day 21 at 250 ppm (see Table D.2.2.6). No adverse gross or microscopic pathological effects were reported in pups.

Table D.2.2.1. Parental male weights in two-generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1 males	Day 0	308.6 ± 30.4	309.5 ± 28.8	312.6 ± 31.0	310.1 ± 29.8	305.9 ± 27.8
	Day 70 (2)	575.3 ± 40.0	585.4 ± 47.1	582.4 ± 55.4	573.5 ± 55.6	484.3 ± 55.8*
	Day 119	636.1 ± 53.4	661.8 ± 58.2	649.5 ± 68.1	633.6 ± 68.5	523.8 66.7*
F1 males	Day 0	59.8 ± 5.4	62.6 ± 7.3	65.6 ± 6.1	58.5 ± 7.4	44.8 ± 6.1*
	Day 105 (2)	592.1 ± 59.9	598.4 ± 64.5	607.6 ± 56.4	581.5 ± 67.2	454.2 ± 47.8*
	Day 154	650.2 ± 64.4	655.9 ± 77.1	670.4 ± 66.3	640.1 ± 73.8	494.8 ± 54.9*

(1) All values in grams: mean ± SD

* Statistically significant by One-way Analysis of Variance and Dunnett's test at alpha = 0.05.

(2) End of premating phase.

Table D.2.2.2. Parental female weights in two-generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1 females	Premating day 0	227.1 ± 17.8	229.7 ± 19.9	228.0 ± 21.5	225.3 ± 15.7	226.0 ± 18.6
	Premating day 70 (2)	313.8 ± 23.1	317.5 ± 28.7	317.8 ± 34.5	300.1 ± 19.5	277.1 ± 23.2*
	Gestation day 0	309.8 ± 20.0	315.0 ± 25.3	317.2 ± 38.3	301.7 ± 20.3	271.3 ± 21.7*
	Gestation day 21	472.4 ± 36.7	466.1 ± 30.9	467.4 ± 45.3	452.8 ± 44.0	407.2 ± 31.9*
	Lactation day 1	354.5 ± 27.0	359.3 ± 22.8	361.8 ± 33.9	342.4 ± 27.7	301.1 ± 22.9*
	Lactation day 21	355.6 ± 23.4	364.0 ± 22.2	368.6 ± 27.1	363.1 ± 30.3	334.7 ± 19.1*
F1 females	Premating day 0	56.7 ± 5.2	58.1 ± 6.0	58.5 ± 5.9	53.8 ± 7.5	42.4 ± 4.7*
	Premating day 105 (2)	323.5 ± 34.1	329.1 ± 47.9	335.2 ± 43.4	315.3 ± 32.8	256.7 ± 23.9*
	Gestation day 0	321.3 ± 34.9	322.4 ± 33.7	336.0 ± 39.2	313.6 ± 30.2	255.6 ± 24.3*
	Gestation day 21	477.5 ± 41.2	471.0 ± 43.6	486.5 ± 49.1	460.9 ± 39.5	388.6 ± 36.4*
	Lactation day 1	373.1 ± 34.4	371.7 ± 45.5	373.0 ± 39.3	356.6 ± 25.4	291.2 ± 24.1*
	Lactation day 21	352.8 ± 30.6	365.7 ± 37.1	362.4 ± 28.4	347.0 ± 32.1	292.9 ± 32.3*

(1) All values in grams: mean ± SD

* Statistically significant by One-way Analysis of Variance and Dunnett's test at alpha = 0.05

(2) End of premating phase.

Table D.2.2.3. Reproductive indices from two-generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1	Mating index (2)	100.0 (30/30)	96.7 (29/30)	100.0 (30/30)	90.0 (27/30)	96.7 (29/30)
	Fertility index (3)	86.7 (26/30)	82.8 (24/29)	83.3 (25/30)	81.5 (22/27)	89.7 (26/29)
F1	Mating index (2)	90.0 (27/30)	90.0 (27/30)	93.3 (28/30)	96.7 (29/30)	96.7 (29/30)
	Fertility index (3)	85.2 (23/27)	63.0 (17/27)	64.3 (18/28)	82.8 (24/29)	96.6 (28/29)

(1) Values in % (numbers in parentheses)
No statistically significant effects were found by One-way Analysis of Variance and Dunnett's test at alpha = 0.05
(2) Mating index = (number copulated/number cohoused) x 100
(3) Fertility index = (number delivered/number copulated) x 100

Table D.2.2.4. Litter data from two-generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
F1 generation	Litter size	14.5 ± 3.58	14.0 ± 3.74	12.8 ± 4.26	13.0 ± 5.96	13.1 ± 3.17
	Live litter size	14.2 ± 3.56	13.8 ± 3.67	12.6 ± 4.08	12.5 ± 6.06	13.0 ± 3.16
	Birth weight (g)	6.8 ± 0.63	7.0 ± 0.63	7.0 ± 0.76	6.8 ± 0.84	7.0 ± 0.96
F2 generation	Litter size	12.3 ± 4.66	13.5 ± 3.81	14.5 ± 1.65	13.3 ± 3.59	12.4 ± 2.89
	Live litter size	12.1 ± 4.49	13.2 ± 3.82	13.8 ± 3.13	13.3 ± 3.57	12.3 ± 2.86
	Birth weight (g)	6.8 ± 0.72	7.0 ± 1.03	6.9 ± 0.86	6.6 ± 0.56	6.7 ± 0.46

(1) Data are mean ± S.D.
No statistically significant effects were found using the Mann-Whitney U test on litter means at alpha = 0.05.

Table D.2.2.5. Viability and lactation indices from two-generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
F1 generation	Viability index (2)	98.4 ± 3.19	99.2 ± 2.22	98.7 ± 2.59	97.8 ± 6.19	98.9 ± 3.50
	Lactation index (3)	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
F2 generation	Viability index (2)	99.4 ± 2.67	97.2 ± 7.32	98.7 ± 3.70	94.5 ± 18.65	98.3 ± 4.78
	Lactation index (3)	99.4 ± 2.67	99.3 ± 3.03	100.0 ± 0.00	95.3 ± 15.99	100.0 ± 0.00

(1) Mean ± S.D.
No statistically significant differences were found using the Mann-Whitney U test on litter means at alpha = 0.05
(2) Viability index = (number alive on postnatal day 4/number alive on postnatal day 0) x 100
(3) Lactation index = (number alive on postnatal day 21/number alive on postnatal day 4 [postcull]) x 100

Table D.2.2.6. Pups weights during lactation from two-generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
F1 generation	Postnatal day 4 (precull)	11.0 ± 1.54	11.5 ± 1.89	12.0 ± 1.93	10.9 ± 2.52	10.3 ± 1.72
	Postnatal day 4 (postcull)	11.1 ± 1.41	11.4 ± 1.92	12.0 ± 1.97	10.9 ± 2.55	10.3 ± 1.75*
	Postnatal day 21	58.9 ± 5.28	61.4 ± 5.49	63.1 ± 4.89	57.7 ± 7.60	44.7 ± 4.76*
F2 generation	Postnatal day 4 (precull)	11.6 ± 2.26	11.2 ± 2.71	11.4 ± 1.17	10.5 ± 2.32	10.1 ± 1.35*
	Postnatal day 4 (postcull)	11.7 ± 2.15	11.4 ± 2.75	11.3 ± 1.08	10.4 ± 2.35	10.1 ± 1.40*
	Postnatal day 21	59.5 ± 6.64	59.8 ± 6.42	60.1 ± 4.82	53.7 ± 6.97*	42.1 ± 5.46*

(1) Data in grams: mean ± S.D
* Significantly different from control using Mann-Whitney U test on litter means at alpha = 0.05

D.3 Acute and Chronic Studies

D.3.1 Acute Inhalation Studies in Rats (IBTL 1972a, 1972b, 1972c)

In this series of tests (IBTL 1972a, 1972b, 1972c) Sprague-Dawley rats were exposed once to fenbutatin oxide by inhalation, and held for 14 days before sacrifice. Results reported included mortality, clinical observations, body weight, and gross pathology of several organs including ovaries. In general, the reports of these studies were minimal.

In the first study (ILBT 1972a), rats were treated with fenbutatin oxide in water aerosol at 0, 0.36, or 1.35 mg fenbutatin oxide/L air (calculated by OEHHA staff from data in the report) for 4 hours. There were 5 rats/sex/group. No deaths occurred, there were no effects on body weight, and no gross pathology of the ovaries was observed.

In the second study (ILBT 1972b), rats were treated with fenbutatin oxide as dust for 4 hours at one of 5 concentrations. The nominal and measured concentrations were 0.11, 0.23, 0.66, 1.1 or 2.3 and 0.048, 0.099, 0.28, 0.46 and 1.0 mg fenbutatin oxide/L air, respectively. There were 5 rats/sex/group. In all except the lowest concentration, deaths occurred among the animals. The incidences among females were 0/5, 1/5, 4/5, 3/5, and 5/5 for the five dose groups. The LC_{50} was calculated as 0.30 mg fenbutatin oxide/L air (this appears to correspond to the measured, as opposed to nominal concentration). Body weight gains were "below normal" for all but the lowest exposure concentration. No gross pathology of the ovaries was observed.

In the third study (ILBT 1972c), rats were treated with fenbutatin oxide as dust for 1 hour at 1.6 or 2.9 mg fenbutatin oxide/L air (nominal concentration). There were 5 rats/sex/group. No deaths occurred. Female weight gains were "below normal" (the mean weight change over 14 days in both exposure groups was a loss). No gross pathology of the ovaries was observed.

D.3.2 Two-year Study in Rats (Shell 1973b)

In this study (Shell 1973b), fenbutatin oxide was administered to male and female Carworth Farm E rats for two years at 0, 50, 100, 300, or 600 ppm in food. A total of 144 control and 72 treated rats per group per sex were used. Subgroups of rats were sacrificed and necropsied at three, six, and twelve months. Twelve controls and six each treated animals/group/sex were sacrificed at three and twelve months, and 24 controls and twelve each treated animals/group/sex were sacrificed at six months. At necropsy, gross and microscopic pathological examinations were made and major viscera were weighed.

Morbidity was similar among males in all groups, but was significantly reduced among females at 600 ppm. Mortality per se was not reported, nor was the observed morbidity further clarified by the authors. Reduced food consumption and weight gain were observed in the early stages of the study among males fed 100, 300 and 600 ppm, and among females fed 300 and 600 ppm. Resulting differences in weight persisted for the duration of the study (see Table D.3.2.1). The authors attributed this to the food being

rendered unpalatable by fenbutatin oxide. Pathological examination found no fenbutatin oxide treatment-related adverse effects on ovaries or uteri.

Table D.3.2.1 Morbidity and body weights for females of two year feeding study in rats (Shell 1973b).

Group		0 ppm	50 ppm	100 ppm	300 ppm	600 ppm
Morbidity (% for two years)		56	48	62	42	33
Body weight (g) [(1)]	Week 0	89	88	90	90	89
	Week 2	141	142	143*	135**	122**
	Week 11	249	246	249	230**	208**
	Week 24	291	288	289	274**	251**
	Week 48	330	330	328	312**	292**
	Week 104	312	307	308	309	292**

(1) Body weight values are means. The authors did not report measures of variation (e.g. standard deviation) for individual means.
* $P < 0.05$ statistically significant difference from controls by Student's t test.
** $P < 0.01$ statistically significant difference from controls by Student's t test.

D.3.3 Eighteen month study in mice (Shell chronic mice, as cited in Shell 1980a and US EPA 1994)

OEHHA staff have not been able to retrieve the original report of this study. Information comes from two secondary sources (Shell 1980a, US EPA 1994) as well as supplemental data to the original study submitted by a later applicant (du Pont 1989a).

In this study, mice were administered fenbutatin oxide at 0, 50, 100, 300, or 600 ppm in food for 18 months. A total of 120 control and 60 treated animals/sex/group were used. Interim sacrifices were performed at 6 and 12 months on 12 controls and 6 treated animals/sex/group (Shell 1980a, US EPA 1994, du Pont 1989a)

The main toxic effect reported was significantly reduced body weight at 300 and 600 ppm (Shell 1980a, US EPA 1994). No hematological, clinical chemical or pathological (gross or histological) adverse effects were found (Shell 1980a). Examination by OEHHA staff of summary tables for gross and histopathological effects (du Pont 1989a) found no adverse effects on ovaries or uterus attributable to fenbutatin oxide treatment at the interim or final sacrifices.

D.3.4 Two-year Study in Dogs (Shell 1973c)

In this study (Shell 1973c, du Pont 1989b), fenbutatin oxide was administered to beagle dogs for two years at 0, 2.5, 5, 15, 30, or 60 mg/kg/d by capsule. There were eight

animals/group/sex in the control group, and four animals/group/sex in the treated groups. Results reported included daily observations for general health and behavior, body weights, clinical chemistry, gross and microscopic pathology at sacrifice, and organ weights.

Vomiting and diarrhea were reported among most animals treated with fenbutatin oxide, with more severe effects at the higher doses. Reductions in body weight were observed at most time points among females exposed to 60 mg/kg/d. This effect was statistically significant only at two years. The authors attributed this effect to vomiting and diarrhea. A statistically significant reduction in body weight at 5 mg/kg/d was attributed by the authors to the skewing influence of one very small animal and not to fenbutatin oxide treatment. The authors of the original report (Shell 1973c) stated that "a wide range of tissues (were) examined microscopically," and that "no... pathological changes were seen in treated or control groups." Examination by OEHHA staff of summary tables for histopathological effects (du Pont 1989b) found no adverse effects on ovaries or uteri attributable to fenbutatin oxide treatment.

Table D.3.4.1. Results for females of two year study in dogs (Shell 1973c)

Group		0	2.5 mg/kg/d	5 mg/kg/d	15 mg/kg/d	30 mg/kg/d	60 mg/kg/d
Number of animals		8	4	4	4	4	4
Body weight (kg) [(1)]	Week 0	8.8	9.8	8.8	8.9	9.1	9.1
	Week 25	12.5	12.5	10.6*	12.5	13.0	11.1
	Week 53	14.4	14.2	11.1*	15.0	14.7	12.2
	Week 104	15.8	15.3	11.6*	15.7	15.2	12.1*

(1) Values are means. The authors did not report measures of variation (e.g. standard deviation) for individual means.

* $P \leq 0.05$ significance of the difference between treatment and control mean by Student's t test.

D.4 Other Relevant Data

Information on the general pharmacokinetics of fenbutatin oxide is presented in Section B.4. No data on the distribution of fenbutatin oxide to female reproductive organs were located.

D.5 Integrative Evaluation

Data on possible female reproductive toxicity come from two multigeneration reproduction studies in rats, supplemented by acute studies in rats and chronic studies in rats, mice, and dogs.

In the earlier rat reproduction study, Long-Evans rats were fed diets with 0, 50, 100, or 300 ppm fenbutatin oxide for three generations, with two litters per generation (Hine Laboratories 1973). In the later study, Crl:CDrBR rats were fed diets with 0, 40, 75, 250, or 300 ppm fenbutatin oxide for two generations with one generation per litter (du Pont 1990). In both studies, no effects on fertility or related indices were found. In the earlier study (Hine Laboratories 1973), litter sizes were consistently reduced at the high concentration, although this was statistically significant for only the F1b litter. In the later study (du Pont 1990), there was no effect on litter size. The later study used larger numbers of animals and was reported in much greater detail than was the earlier study. However, comparison is complicated by the fact that the two studies were conducted in different strains of rats. The earlier study did not report birth weights; no effect on birth weights was found in the later study. Parental weights were reduced at the high concentration in both studies.

In the earlier rat reproduction study (Hine Laboratories 1973), reduced postnatal survival was observed in both litters of the third generation. Reduced pup weight at weaning was consistently found. In the later rat reproduction study (du Pont 1990), no effect on pup survival was found. It should be noted that this was a two generation study, so cannot be directly compared to the finding of reduced pup survival in the third generation of the earlier study. Pup body weights were reduced during lactation in the later study.

It is possible that the effects on lactating pups seen in the rat reproductive studies were due to problems with lactation in the dams. However, there are no data specifically supporting this possibility. It is also possible that the effects on lactating pups were due to transfer of fenbutatin oxide through the milk, or from pups eating their mother's food, or that the effects resulted from prenat exposure, but were manifested postnatally. As noted in section C.5, as the statute is currently interpreted, developmental effects resulting from postnatal exposure are outside the purview of Proposition 65 (OEHHA 1996).

Gross and/or histopathological examinations found no effects on ovaries or uteri attributable to fenbutatin oxide treatment in three acute studies in rats, or chronic studies in rats, mice, or dogs.

E. MALE REPRODUCTIVE TOXICITY

E.1 Overview

Data on the male reproductive toxicity of fenbutatin oxide comes from two multigeneration studies in rats, a mouse dominant lethal study, acute and subchronic studies in rats, and chronic studies in rats, mice, and dogs. No studies concerning human exposure and reproductive effects were located.

E.2 Multigeneration Studies (Rats)

In these studies, fenbutatin oxide was administered to both sexes. It is therefore sometimes difficult to distinguish potential male reproductive effects from female reproductive effects. Those effects which are often associated with male reproductive toxicity (e.g. fertility, litter size, testis weights) are discussed in this section. A more in depth discussion, including female reproductive effects, is presented above (section D.2).

E.2.1 Three Generation Reproduction Study (Hine Laboratories 1973)

In the earlier reproduction study (Hine Laboratories 1973), Long-Evans rats were fed 0, 50, 100 or 300 ppm fenbutatin oxide for 3 generations, with 2 litters per generation. There were 20 females and 10 males per group. The second litter of each generation became the parental group of the next generation. Litters were culled to 10 on postnatal day (pnd) 5. Parental endpoints reported included survival, body weight, clinical observations and gross pathology. Reproductive endpoints reported included fertility, litter size, postnatal survival, postnatal (but not birth) weights, and selected pup organ weights and histopathology.

No parental deaths were reported. Parental body weights at sacrifice were consistently reduced at 300 ppm (statistically significant for females in 2 out of 3 generations, for males in all generations). The magnitude of the reduction in weight was 6-8% for females and 10-14% for males (see Table E.2.1.1). Hyperactivity and irritability were also reported at 300 ppm.

Results for male fertility (male fertility index) were not reported. Fertility on a per female basis ranged from 90% to 100% and was not affected by fenbutatin oxide treatment. There were 10 males and 20 females per treatment group; the mating procedure was not described in the report. No effects on gestation indices (number of litters with live pups/number of pregnancies) were observed. Litter sizes were similar, although the 300 ppm group litter size was consistently smaller than controls. These differences were statistically significant only for the F1b litter (see Table E.2.1.2). Birth weights were not reported. For the F3b weanlings, the relative testes weight was reduced (statistically significant) at 100 and 300 ppm. No effects on relative brain, heart, liver, or kidney weights were observed (see Table E.2.1.3).

Table E.2.1.1. Parental weights at sacrifice from three-generation rat reproductive study (Hine Laboratories 1973). (1)

sex	Female weight at sacrifice				Male weight at sacrifice			
group	Control	50 ppm	100 ppm	300 ppm	Control	50 ppm	100 ppm	300 ppm
F0	357 ± 7.7	360 ± 7.1	366 ± 6.6	328 ± 6.9*	556 ± 14.9	542 ± 16.4	571 ± 8.4	478 ± 14.7**
F1b	361 ± 8.7	381 ± 6.8	384 ± 7.5	334 ± 5.3*	514 ± 24.6	544 ± 16.4	514 ± 17.4	442 ± 13.1*
F2b	338 ± 9.7	362 ± 6.2	352 ± 5.8	316 ± 7.9	524 ± 26.5	529 ± 12.2	541 ± 15.4	469 ± 11.0*

(1) Mean ± SE

* Significant for P = 0.05 by Dunnett's test.

** Significant for P = 0.01 by Dunnett's test.

Table E.2.1.2. Litter sizes from three-generation rat reproductive study (Hine Laboratories 1973). (1)

group	Control	50 ppm	100 ppm	300 ppm
F1a	11.0 ± 0.56	10.8 ± 0.47	10.8 ± 0.41	9.7 ± 0.47
F1b	12.3 ± 0.45	11.4 ± 0.69	11.5 ± 0.45	9.7 ± 0.58**
F2a	10.9 ± 0.31	11.6 ± 0.32	11.5 ± 0.39	10.0 ± 0.47
F2b	11.6 ± 0.54	11.6 ± 0.63	10.6 ± 0.63	10.8 ± 0.37
F3a	10.0 ± 0.41	10.5 ± 0.38	10.1 ± 0.39	9.1 ± 0.29
F3b	11.8 ± 0.34	11.8 ± 0.42	11.8 ± 0.41	10.5 ± 0.50

(1) Mean ± SE

** Significant for p = 0.01 by Dunnett's test.

The number of females bearing litters ranged from 18 to 20 per group, and was not affected by fenbutatin oxide treatment.

Table E.2.1.3. Body weights for F3b weanlings in three-generation reproductive study (Hine Laboratories 1973). (1)

group	0	50 ppm	100 ppm	300 ppm
Body weight (g)	33.4 ± 1.05	31.2 ± 0.59	32.4 ± 0.58	29.3 ± 1.46*
Number of litters	19	18	18	15

(1) Data for males and females combined.
Mean ± SE.
* Significant at P = 0.05 by Dunnett's test

Table E.2.1.4. Relative organ weights for F3b weanlings in three-generation reproductive study (Hine Laboratories 1973). (1)

Organ	males				females			
	0	50 ppm	100 ppm	300 ppm	0	50 ppm	100 ppm	300 ppm
Brain	3.76 ± 0.051	3.60 ± 0.054	3.71 ± 0.049	3.75 ± 0.118	3.58 ± 0.060	3.54 ± 0.050	3.56 ± 0.062	3.80 ± 0.139
Heart	0.65 ± 0.022	0.65 ± 0.033	0.67 ± 0.046	0.65 ± 0.019	0.66 ± 0.016	0.66 ± 0.020	0.68 ± 0.035	0.68 ± 0.021
Liver	4.16 ± 0.076	4.15 ± 0.158	4.04 ± 0.152	4.06 ± 0.034	4.29 ± 0.056	4.46 ± 0.017	4.25 ± 0.074	(2)
Kidney	1.29 ± 0.027	1.25 ± 0.048	1.29 ± 0.029	1.25 ± 0.026	1.31 ± 0.026	1.33 ± 0.031	1.26 ± 0.034	1.38 ± 0.046
Testes	0.38 ± 0.015	0.38 ± 0.014	0.32 ± 0.014*	0.32 ± 0.007 **	NA	NA	NA	NA
Number of animals	10	5	5	10	10	5	5	10

(1) Mean ± SE, except for numbers of animals.
Organ weights expressed as (organ weight/body weight) x 100
* Significant at P = 0.05 by Dunnett's test
**Significant at P = 0.01 by Dunnett's test
(2) Data lost in copying.

E.2.2 Two-Generation Reproduction Study (du Pont 1990)

In the later reproduction study, Crl:CDrBR rats were fed 0, 40, 75, 250, or 500 ppm fenbutatin oxide for 2 generations, with 1 litter per generation. There were 30 females and 30 males per group. Parental endpoints reported included survival, body weight, food consumption and clinical observations. Reproductive endpoints reported included histopathology of parental reproductive tissues, mating, gestation, and fertility indices, litter size, birth weight, postnatal survival and weight, and gross and microscopic pathology of pups.

In the P1 generation, 1 male rat in the 500 ppm group died. In the F1 generation, 2 males in the 500 ppm group, 1 male in the 250 ppm group, 1 female in the 500 ppm group, 1 female in the 75 ppm group, and 2 females in the control group died. The authors concluded that none of the deaths were attributable to fenbutatin oxide treatment. Parental (male and female) body weights and weight gains during the premating and gestation periods were consistently reduced at 500 ppm (see Tables E.2.2.1 and E.2.2.2). Male body weights at the end of the premating phase were reduced (statistically significant) by approximately 16% (P1) and 23% (F1) at 500 ppm compared to controls. Among P1 females at 500 ppm, mean body weights were approximately 12% lower than controls on day 0 of gestation, and 14% lower on day 21 of gestation; these reductions were statistically significant. Among P1 males, no effects on absolute testes weights were observed, although at 500 ppm relative testes weights were increased (statistically significant). Among F1 males at 500 ppm, absolute testes weights were reduced (statistically significant), and relative testes weight was increased (statistically significant) (see Table E.2.2.3). No adverse clinical observations or gross or microscopic pathological effects were reported.

Breeding was performed one male to one female. No effects on mating index (number copulating/number cohoused) were observed. No effect on the fertility index (number delivering/number copulating) was observed in the P1 generation. The fertility index was lower for treated than control animals (not statistically significant) in the F1 females at 40 and 75 ppm, but not at 250 or 500 ppm (see Table E.2.2.4). No effects on litter size or birth weight were observed (see Table E.2.2.5). No adverse gross or microscopic pathological effects were reported in pups.

Table E.2.2.1. Parental male weights in two-generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1 males	Day 0	308.6 ± 30.4	309.5 ± 28.8	312.6 ± 31.0	310.1 ± 29.8	305.9 ± 27.8
	Day 70 (2)	575.3 ± 40.0	585.4 ± 47.1	582.4 ± 55.4	573.5 ± 55.6	484.3 ± 55.8*
	Day 119	636.1 ± 53.4	661.8 ± 58.2	649.5 ± 68.1	633.6 ± 68.5	523.8 66.7*
F1 males	Day 0	59.8 ± 5.4	62.6 ± 7.3	65.6 ± 6.1	58.5 ± 7.4	44.8 ± 6.1*
	Day 105 (2)	592.1 ± 59.9	598.4 ± 64.5	607.6 ± 56.4	581.5 ± 67.2	454.2 ± 47.8*
	Day 154	650.2 ± 64.4	655.9 ± 77.1	670.4 ± 66.3	640.1 ± 73.8	494.8 ± 54.9*

(1) All values in grams: mean ± SD

* Statistically significant by One-way Analysis of Variance and Dunnett's test at alpha = 0.05.

(2) End of premating phase.

Table E.2.2.2. Parental female weights in two-generation rat reproductive study (du Pont 1990)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1 females	Premating day 0	227.1 ± 17.8	229.7 ± 19.9	228.0 ± 21.5	225.3 ± 15.7	226.0 ± 18.6
	Premating day 70 (2)	313.8 ± 23.1	317.5 ± 28.7	317.8 ± 34.5	300.1 ± 19.5	277.1 ± 23.2*
	Gestation day 0	309.8 ± 20.0	315.0 ± 25.3	317.2 ± 38.3	301.7 ± 20.3	271.3 ± 21.7*
	Gestation day 21	472.4 ± 36.7	466.1 ± 30.9	467.4 ± 45.3	452.8 ± 44.0	407.2 ± 31.9*
	Lactation day 1	354.5 ± 27.0	359.3 ± 22.8	361.8 ± 33.9	342.4 ± 27.7	301.1 ± 22.9*
	Lactation day 21	355.6 ± 23.4	364.0 ± 22.2	368.6 ± 27.1	363.1 ± 30.3	334.7 ± 19.1*
F1 females	Premating day 0	56.7 ± 5.2	58.1 ± 6.0	58.5 ± 5.9	53.8 ± 7.5	42.4 ± 4.7*
	Premating day 105 (2)	323.5 ± 34.1	329.1 ± 47.9	335.2 ± 43.4	315.3 ± 32.8	256.7 ± 23.9*
	Gestation day 0	321.3 ± 34.9	322.4 ± 33.7	336.0 ± 39.2	313.6 ± 30.2	255.6 ± 24.3*
	Gestation day 21	477.5 ± 41.2	471.0 ± 43.6	486.5 ± 49.1	460.9 ± 39.5	388.6 ± 36.4*
	Lactation day 1	373.1 ± 34.4	371.7 ± 45.5	373.0 ± 39.3	356.6 ± 25.4	291.2 ± 24.1*
	Lactation day 21	352.8 ± 30.6	365.7 ± 37.1	362.4 ± 28.4	347.0 ± 32.1	292.9 ± 32.3*

(1) All values in grams: mean ± SD

* Statistically significant by One-way Analysis of Variance and Dunnett's test at alpha = 0.05

(2) End of premating phase.

Table E.2.2.3. Parental male testes and final body weights in two-generation rat reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1 males	Absolute testes weight (g)	3.637 ± 0.361	3.664 ± 0.555	3.689 ± 0.372	3.614 ± 0.440	3.647 ± 0.352
	Relative testes weight (% of body weight)	0.5628 ± 0.0790	0.5455 ± 0.0785	0.5629 ± 0.0624	0.5668 ± 0.0882	0.6938 ± 0.0734*
	Final body weight (g)	651.2 ± 52.0	673.2 ± 59.1	659.2 ± 67.6	644.5 ± 70.9	529.7 ± 64.8*
F1 males	Absolute testes weight (g)	4.036 ± 0.353	4.025 ± 0.466	4.076 ± 0.346	3.838 ± 0.453	3.776 ± 0.310*
	Relative testes weight (% of body weight)	0.6037 ± 0.0788	0.6003 ± 0.814	0.5947 ± 0.0716	0.5932 ± 0.0644	0.7444 ± 0.0935*
	Final body weight (g)	675.1 ± 68.7	676.3 ± 74.6	691.0 ± 65.2	654.0 ± 96.9	512.6 ± 59.0*

(1) Mean ± SD

* Significantly different ($P < 0.05$) from control group by Dunnett's test.

Table E.2.2.4. Reproductive indices from two-generation reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
P1	Mating index (2)	100.0 (30/30)	96.7 (29/30)	100.0 (30/30)	90.0 (27/30)	96.7 (29/30)
	Fertility index (3)	86.7 (26/30)	82.8 (24/29)	83.3 (25/30)	81.5 (22/27)	89.7 (26/29)
F1	Mating index (2)	90.0 (27/30)	90.0 (27/30)	93.3 (28/30)	96.7 (29/30)	96.7 (29/30)
	Fertility index (3)	85.2 (23/27)	63.0 (17/27)	64.3 (18/28)	82.8 (24/29)	96.6 (28/29)

(1) Values in % (numbers in parentheses)
No statistically significant effects were found by One-way Analysis of Variance and Dunnett's test at alpha = 0.05
(2) Mating index = (number copulated/number cohoused) x 100
(3) Fertility index = (number delivered/number copulated) x 100

Table E.2.2.5. Litter data from two-generation reproductive study (du Pont 1990). (1)

Group		0 ppm	40 ppm	75 ppm	250 ppm	500 ppm
F1 generation	Litter size	14.5 ± 3.58	14.0 ± 3.74	12.8 ± 4.26	13.0 ± 5.96	13.1 ± 3.17
	Live litter size	14.2 ± 3.56	13.8 ± 3.67	12.6 ± 4.08	12.5 ± 6.06	13.0 ± 3.16
	Birth weight (g)	6.8 ± 0.63	7.0 ± 0.63	7.0 ± 0.76	6.8 ± 0.84	7.0 ± 0.96
F2 generation	Litter size	12.3 ± 4.66	13.5 ± 3.81	14.5 ± 1.65	13.3 ± 3.59	12.4 ± 2.89
	Live litter size	12.1 ± 4.49	13.2 ± 3.82	13.8 ± 3.13	13.3 ± 3.57	12.3 ± 2.86
	Birth weight (g)	6.8 ± 0.72	7.0 ± 1.03	6.9 ± 0.86	6.6 ± 0.56	6.7 ± 0.46

(1) Data are mean ± S.D.
No statistically significant effects were found using the Mann-Whitney U test on litter means at alpha = 0.05.

E.3 Dominant Lethal Study in Mice (Shell 1972)

In a dominant lethal study in mice (Shell 1972), fenbutatin oxide was administered once orally to males at 0, 250, or 500 mg/kg in 1% carboxymethyl cellulose. Endoxan was administered once orally at 200 mg/kg as a positive control. There were 16 animals in the control group and 8 animals each in the treated groups. Following administration, treated and control males were caged with 3 untreated females each for one week. This was repeated with new groups of females for 8 weeks. Females were sacrificed on presumed gestation day 13, and contents of the uterus examined.

No male deaths occurred. The authors did not report results for other indications of male toxicity. The dose selection rationale was not given, and it is not apparent from the data in this report whether the doses resulted in systemic toxicity.

No statistically significant effects of fenbutatin oxide treatment on number of pregnancies, total number of implants, or early fetal deaths were observed (see Tables E.3.1 to E.3.3). Endoxan treated animals were observed to have reduced pregnancies and fetal implants, and increased early fetal deaths on various weeks.

Table E.3.1. Percentage of pregnancies in mated mice (Shell 1972). (1)

Time of mating (weeks after dosing)	Fenbutatin oxide			Endoxan 200 mg/kg
	0	250 mg/kg	500 mg/kg	
1	67	63	79	71
2	71	75	75	42**
3	73	67	67	46**
4	71	54	63	54
5	50	67	58	46
6	67	75	79	46
7	71	67	63	42**
8	60	58	58	33*

(1) The authors did not report measures of variation (e.g. standard deviation).
Statistical significance tested using chi-square test.
* Significant difference from control $P < 0.05$
** Significant difference from control $P < 0.01$

Table E.3.2. Mean total fetal implants per pregnant female (Shell 1972). [1]

Time of mating (weeks after dosing)	Fenbutatin oxide			Endoxan 200 mg/kg
	0	250 mg/kg	500 mg/kg	
1	12.6	11.8	12.1	8.0***
2	13.0	12.6	11.4	9.1***
3	12.5	12.5	13.1	12.7
4	11.4	12.3	12.7	12.5
5	12.3	10.6	11.7	11.2
6	11.5	12.9	12.4	12.3
7	11.8	11.6	12.8	11.1
8	13.1	12.9	12.1	11.8

(1) The authors did not report measures of variation (e.g. standard deviation).
Statistical significance tested using 2-way ANOVA.
*** Significant difference from control at $P < 0.001$.

Table E.3.3. Mean early fetal deaths per pregnant female (Shell 1972). [1]

Time of mating (weeks after dosing)	Fenbutatin oxide			Endoxan 200 mg/kg
	0	250 mg/kg	500 mg/kg	
1	0.96	1.11	1.04	1.75***
2	1.03	0.91	0.94	1.60***
3	0.96	0.99	0.77	1.56***
4	0.87	1.18	1.18	1.08
5	0.91	1.02	0.95	0.77
6	1.07	0.97	0.91	1.26
7	1.00	0.86	0.91	1.23
8	0.87	0.97	0.84	1.08

(1) The authors did not report measures of variation (e.g. standard deviation).
Statistical significance tested using 2-way ANOVA on transformed data.
*** Significant difference from control at $P < 0.001$.

E.4 Acute, Subchronic and Chronic Studies

E.4.1 Acute Inhalation Studies in Rats (IBTL 1972a, 1972b, 1972c)

In this series of tests (IBTL 1972a, 1972b, 1972c) Sprague-Dawley rats were exposed once to fenbutatin oxide by inhalation and held for 14 days before sacrifice. Results reported included mortality, clinical observations, body weight, and gross pathology of several organs including testes. In general, the reports of these studies were minimal, and evaluation is difficult.

In the first study (ILBT 1972a), rats were treated with fenbutatin oxide in water aerosol at 0, 0.36, or 1.35 mg fenbutatin oxide/L air (calculated by OEHHA staff from data in the report) for 4 hours. There were 5 rats/sex/group. No deaths occurred, there were no effects on body weight, and no gross pathology of the testes was observed.

In the second study (ILBT 1972b), rats were treated with fenbutatin oxide as dust for 4 hours at one of 5 concentrations. The nominal and measured concentrations were 0.11, 0.23, 0.66, 1.1 or 2.3 and 0.048, 0.099, 0.28, 0.46 and 1.0 mg fenbutatin oxide/L air, respectively. There were 5 rats/sex/group. In all except the lowest concentration, deaths occurred among the animals. The incidences among males were 0/5, 0/5, 2/5, 4/5, and 4/5 for the five dose groups. The LC50 was calculated as 0.30 mg fenbutatin oxide/L air (this appears to correspond to the measured, as opposed to nominal concentration). Body weight gains were "below normal" for all but the lowest exposure concentration. No gross pathology of the testes was observed.

In the third study (ILBT 1972c), rats were treated with fenbutatin oxide as dust for 1 hour at 1.6 or 2.9 mg fenbutatin oxide/L air (nominal concentration). There were 5 rats/sex/group. No deaths occurred, male weight gains were "within normal limits," and no gross pathology of the testes was observed.

E.4.2 One Month Study in Rats (SRI 1970)

This study (SRI 1970) was initially conducted to examine the palatability of 3 organo-tin compounds, including fenbutatin oxide, but was extended when certain toxic manifestations became apparent. Male Long-Evans rats (5 per group) were treated with fenbutatin oxide or one of 2 other organo-tin compounds at 0, 250, 500, or 1000 ppm in food for 1 month. The age at which treatment was started was not reported. Observations included general health, food consumption, several organ weights (including testes), and gross and microscopic pathology.

No deaths were observed among animals treated with fenbutatin oxide. Mild diarrhea was observed in all treatment groups during the first week. Decreased activity and occasional prostration was observed at 500 and 1000 ppm. There were dose-related reductions in body weight gain and total food consumption across the 28-day treatment period which were statistically significant at 1000 ppm. A dose-related increase (statistically significant) in relative testicular weights at 500 and 1000 ppm was observed. OEHHA staff calculation indicates that absolute testes weight also increased at these concentrations. The statistical significance of the differences in absolute testes weights has not been addressed (see Table E.4.2.1). The other two organo-tin compounds also produced increased relative testicular weight. Pathological examination found no fenbutatin oxide treatment related adverse gross or microscopic effects on organs (including testes).

Table E.4.2.1. Results from one month study in rats (SRI 1970). [1]

Group	0 ppm	250 ppm	500 ppm	1000 ppm
Number of animals	5	5	5	5
Body weight day 0 (g)	245	221	232	224
Body weight day 28 (g)	348	317	320	300
Body weight gain days 0-28 (g)	103	96	88	76*
Food consumption days 0-28 (g)	647	616	606	533*
Relative heart weight [2]	0.30	0.34	0.38	0.36
Relative liver weight [2]	4.36	4.41	4.54	4.31
Relative kidney weight [2]	0.95	0.92	1.02	0.89
Relative spleen weight [2]	0.14	0.15	0.23*	0.20
Relative testes weight [2]	0.89	0.92	1.01*	1.10*
Absolute testes weight (g) [3]	3.10	2.92	3.23	3.30

(1) Values are means, except for numbers of animals. The authors did not report measures of variation (e.g. standard deviation).
* Significantly different from controls using Student's t-test at $p < 0.05$.
(2) g/100 g body weight
(3) Calculated by OEHHA from body weight and relative testes weight. The statistical significance of differences was not addressed.

E.4.3 Two-year Study in Rats (Shell 1973b)

In this study (Shell 1973b), fenbutatin oxide was administered to male and female Carworth Farm E rats for 2 years at 0, 50, 100, 300, or 600 ppm in food. Subgroups of rats were sacrificed and necropsied at 3, 6, and 12 months. A total of 144 control and 72 treated rats per group per sex were used. At necropsy, gross and microscopic pathological examinations were made and major viscera were weighed.

Morbidity was similar among all male groups, but significantly reduced among females at 600 ppm. Mortality per se was not reported, nor was the observed morbidity further clarified by the authors. Reduced food consumption and growth were observed in the early stages of the study among males fed 100, 300 and 600 ppm, and among females fed 300 and 600 ppm. Resulting differences in weight persisted for the duration of the study (see Table E.4.3.1). The authors attributed this to the food being rendered unpalatable by fenbutatin oxide. There were no effects on absolute testes weight at 3, 6 or 12 months. Adjusted testes weights were increased (statistically significant) at 600 ppm at 3 months. Adjusted testes weights were not calculated by the authors for 6 or 12 month sacrifices. After 2 years, absolute testes weights were increased in a dose-related manner. This increase was statistically significant at 600 ppm. Adjusted testes weights were also increased in a dose-related manner. This increase was statistically significant at 300 and 600 ppm. Relative testes weights (calculated by OEHHA staff) showed a similar dose-related increase (see Table E.4.3.2). Pathological examination found no fenbutatin oxide treatment related testicular hypertrophy or pathology.

Table E.4.3.1 Morbidity and body weights for males of two year feeding study in rats (Shell 1973b). (1)

Group		0 ppm	50 ppm	100 ppm	300 ppm	600 ppm
Morbidity (% for two years)		67	60	54	71	65
Body weight (g) (1)	Week 0	103	105	103	106	102
	Week 2	178	170**	164**	171**	145**
	Week 12	376	374	362**	362**	326**
	Week 24	446	441	431**	428**	396**
	Week 48	502	505	488**	489**	456**
	Week 104	402	369*	374	377	361**

(1) Body weight values are means. The authors did not report measures of variation (e.g. standard deviation) for individual means.
* $P < 0.05$ statistically significant difference from controls by Student's t test.
** $P < 0.01$ statistically significant difference from controls by Student's t test.

Table E.4.3.2 Testes weights for males of two year feeding study in rats (Shell 1973b). [1]

Group		0 ppm	50 ppm	100 ppm	300 ppm	600 ppm
3 month sacrifice	Number of animals	12	6	6	6	6
	Body weight	375	385	367	352*	326**
	Absolute testes weight	3.54	3.50	3.50	3.54	3.45
	Relative testes weight [2]	0.944	0.909	0.954	1.006	1.058
	Adjusted testes weight [3]	3.43	3.40	3.48	3.60	3.73*
6 month sacrifice	Number of animals	24	11	12	12	12
	Body weight	459	463	461	450	405*
	Absolute testes weight	3.69	3.81	3.82	3.84	3.66
	Relative testes weight [2]	0.804	0.823	0.829	0.853	0.904
12 month sacrifice	Number of animals	11	6	5	6	6
	Body weight	493	492	478	481	427*
	Absolute testes weight	3.85	4.02	3.82	4.00	3.84
	Relative testes weight [2]	0.781	0.817	0.799	0.832	0.899
2 year sacrifice	Number of animals	32/31	19/19	22/22	14/14	17/16
	Body weight	390	383	386	380	378
	Absolute testes weight	3.60	3.74	4.02	4.30	4.56*
	Relative testes weight [2]	0.923	0.976	1.041	1.132	1.206
	Adjusted testes weight [4]	3.52	3.80	3.94	4.42*	4.65**

(1) Values are means, except for numbers of animals. The authors did not report measures of variation (e.g. standard deviation) for individual means.
* Statistically significant $P < 0.05$ by Students t-test.
** Statistically significant $P < 0.01$ by Students t-test.
(2) Calculated by OEHHA staff (g testes weight/100 g body weight).
(3) Adjusted for differences in terminal body weight (method not specified by authors).
(4) Adjusted for differences in initial and terminal body weight (method not specified by authors).

E.4.4 Eighteen month study in mice (Shell chronic mice, as cited in Shell 1980a and US EPA 1994)

OEHHA staff have not been able to retrieve the original report of this study. Information comes from two secondary sources (Shell 1980a, US EPA 1994) and supplemental data from the original study submitted by a later applicant (du Pont 1989a).

In this study, mice were administered fenbutatin oxide at 0, 50, 100, 300, or 600 ppm in food for 18 months. A total of 120 control and 60 treated animals/sex/group were used. Interim sacrifices were performed at 6 and 12 months on 12 controls and 6 treated animals/sex/group (Shell 1980a, US EPA 1994, du Pont 1989a)

The main toxic effect reported was significantly reduced body weight at 300 and 600 ppm (Shell 1980a, US EPA 1994). No hematological, clinical chemical or pathological (gross or histological) adverse effects were found (Shell 1980a). Examination by OEHHA staff of summary tables for histopathological effects (du Pont 1989a) found no adverse effects on the testes attributable to fenbutatin oxide treatment at the interim or final sacrifices.

E.4.5 Two-year Study in Dogs (Shell 1973c)

In this study (Shell 1973c, du Pont 1989b), fenbutatin oxide was administered to beagle dogs for two years at 0, 2.5, 5, 15, 30, or 60 mg/kg/d by capsule. There were 8 animals/group/sex in the control group, and 4 animals/group/sex in the treated groups. Results reported included daily observations for general health and behavior, body weights, clinical chemistry, gross and microscopic pathology at sacrifice, and organ weights. Vomiting and diarrhea were reported among most animals treated with fenbutatin oxide, with more severe effects at the higher doses. Statistically significant reductions in body weight were observed at most time points among males exposed to 60 mg/kg/d. The authors attributed this to vomiting and diarrhea. There were also dose-related, but not statistically significant, reductions in body weight at lower doses. Among males, the relative testes weight was significantly increased at 60 mg/kg/d. However, the absolute testes weight for was only slightly higher than other groups, and there was no dose-related trend (See Table E.4.5.1). The authors of the original report (Shell 1973c) stated that "a wide range of tissues (were) examined microscopically," and that "no... pathological changes were seen in treated or control groups." Examination by OEHHA staff of summary tables for histopathological effects (du Pont 1989b) found no adverse effects on testes attributable to fenbutatin oxide treatment.

Table E.4.5.1. Results for males of two year study in dogs (Shell 1973c). [1]

Group		0	2.5 mg/kg/d	5 mg/kg/d	15 mg/kg/d	30 mg/kg/d	60 mg/kg/d
Number of animals		8	4	4	4	4	4
Body weight (kg)	Week 0	10.1	10.3	10.1	10.2	10.1	9.8
	Week 25	14.4	14.5	14.5	13.9	13.7	12.1*
	Week 53	17.1	16.8	16.9	15.9	15.2	14.5*
	Week 104	18.4	16.5	18.0	16.5	15.7	15.4
Absolute testes weight (104 weeks) (g)		22.72	22.56	20.75	22.05	19.76	23.18
Relative testes weight (104 weeks) (g/100 g body weight)		0.126	0.133	0.119	0.132	0.126	0.156*

(1) Values are means, except for numbers of animals. The authors did not report measures of variation (e.g. standard deviation) for individual means.
* $P \leq 0.05$ significance of the difference between treatment and control mean by Student's t test.

E.5 Other Relevant Data

E.5.1 Pharmacokinetics

Information on the general pharmacokinetics of fenbutatin oxide is presented in section B.4. No data on the distribution of fenbutatin oxide to male reproductive organs were located.

E.5.2 Effects of Feed Restriction on Male Rat Reproductive Endpoints

In most of the rat studies cited above, the highest treatment level elicited some reduction in food consumption and weight or weight gain. Data on the effects of feed restriction and reduced weight on male reproductive parameters in Sprague-Dawley rats is available from a study by Chapin et al. (1993). Adult male rats were feed restricted such that body weight was maintained at about 90%, 80%, or 70% of controls for 15 weeks . Rats were bred at 8 and 15 weeks, and sacrificed for examination after 15 weeks. At the levels of feed restriction examined, there was no effect on fertility (litters/male), litter size, proportion of pups born alive, or adjusted live pup weight at 8 or 15 weeks. There was no effect on absolute weights of cauda epididymis, epididymis, or testes. As a result, relative testes weight was increased in a treatment-related manner. This increase was statistically significant at all treatment levels (see table E.5.2.1). There was a treatment-related, statistically significant reduction in absolute prostate and seminal vesicle weight at all feed restriction levels. Epididymal sperm density and total spermatid heads/testes were unaffected by treatment. Epididymal sperm motility was statistically significantly reduced at all treatment levels. However, there was no treatment-related trend. Abnormal sperm were not increased.

Table E.5.2.1. Body and testes weights from 15 week feed restriction study in rats (Chapin et al. 1993). (1)

Group	100%	90%	80%	70%
Body weight (g)	670.8 ± 13.4	592.8 ± 5.3	536.8 ± 3.5	468.5 ± 5.7
Absolute right testis weight (g)	1.89 ± 0.03	1.87 ± 0.03	1.85 ± 0.02	1.95 ± 0.03
Relative right testis weight (g/100g body weight) (2)	0.282	0.315*	0.344*	0.416*
Number of animals	20	20	20	20

(1) values are mean ± S.E.M., except for number of animals and relative testes weight (mean only).
(2) Relative testes weight calculated by OEHHA staff. Statistical significance calculated by authors (Chapin et al.).
* Significantly different from 100% group, $p < 0.05$ by Dunn's test.

E.6. Integrative Evaluation

Data on possible reproductive toxicity come from two multigeneration reproductive toxicity studies in rats, a dominant lethal study in mice, acute and subchronic studies in rats, and chronic studies in rats, mice, and dogs.

In the earlier rat reproductive toxicity study, Long-Evans rats were fed diets with 0, 50, 100, or 300 ppm fenbutatin oxide for three generations, with two litters per generation (Hine Laboratories 1973). In the later study, Crl:CDrBR rats were fed diets with 0, 40, 75, 250, or 300 ppm fenbutatin oxide for two generations with one generation per litter (du Pont 1990). In these studies, both sexes were treated. No effects on fertility or related indices were found. In the earlier study (Hine Laboratories 1973), litter sizes were consistently reduced at the high concentration, although this was statistically significant for only the F1b litter. It is possible that the effect on litter size was the result of a male mediated effect. In the later study (du Pont 1990), there was no effect on litter size. The later study used larger numbers of animals and was reported in much greater detail than the earlier study. However, comparison is complicated by the fact that the two studies were conducted in different strains of rats.

No evidence for dominant lethal or other reproductive effects was found in a dominant lethal study in mice. However, it is not clear whether a systemically toxic dose was used.

There is some evidence of effects of fenbutatin oxide treatment on testes weight in rats. In a one month study, both relative and absolute testes weights were increased. Relative weights were increased in a dose-related manner at all doses. However, body weights were decreased in a similar manner. OEHHA staff calculations indicate that absolute testes weights were increased somewhat at the two highest concentrations tested. The statistical significance of the differences has not been assessed (SRI 1970: see section E.4.2). In a two year study, both relative and absolute testes weights were increased at termination. However, absolute testes weights were not increased at the 3, 6, or 12 month sacrifices. Less than half of the animals survived the full two years of the study (Shell 1973b: see E.4.3).

Effects in the opposite direction were found in the offspring of treated rats. In a three-generation reproductive toxicity study, the relative testes weights of F3b weanlings were reduced (Hine Laboratories 1973: see section E.2.1). In a later, two-generation rat reproductive toxicity study, there was no effect of treatment on the absolute testes weight of the mature P1 animals, although relative testes weight was increased together with decreased body weight. For mature animals in the F1 generation, absolute testes weights were significantly reduced at the high concentration. Body weight was also significantly reduced, and relative testes weight was significantly increased (du Pont 1990: see section E.2.2).

In a 15 week feed restriction study in rats, feed was adjusted to maintain body weight at approximately 90%, 80%, or 70% of control (ad lib) weight. Absolute testes weights were not affected, but relative testes weights were increased statistically significantly together with reduced body weight (Chapin et al. 1993: see section E.5.2).

In a two year study in dogs, absolute testes weights were similar at all concentrations of fenbutatin oxide tested. Relative testes weights were increased at the high concentration,

together with reduced body weight (Shell 1973c: see section E.4.5). OEHHA staff have not been able to retrieve the testes weight data from an 18 month mouse study.

Gross and/or histopathological examinations found no adverse effects on testes attributable to fenbutatin oxide treatment in three acute studies in rats, a subchronic study in rats, or chronic studies in rats, mice, or dogs.

F. Summary

F.1 Developmental Toxicity

Data on the possible developmental toxicity of fenbutatin oxide come primarily from one rat and three rabbit developmental studies (Shell 1973a, 1980b, 1981), supplemented with results from two multigeneration studies in rats.

In the rat developmental study (Shell 1980b), there were reductions in the percentage of animals with evidence of pregnancy at termination at the mid and high doses (30 and 60 mg/kg/d). This was statistically significant ($p = 0.025$) at the mid dose, and marginally significant at the high dose ($p = 0.055$). There was a statistically significant increase in pre-implantation losses at the high dose compared to controls (Wilcoxon rank sum test); however, in terms of mean loss, differences between control and treated animals were small, with mean loss greater at the low dose than the high dose and the mid dose not differing from controls. Thus, mean losses showed no clear dose-response trend. Additionally, there were several animals where the number of implants exceeded the number of corpora lutea.

The statistically significant increase in pre-implantation losses was one of the effects referred to in the citation supporting addition of fenbutatin oxide to the TRI list (US EPA 1994a).

In this study, exposure to fenbutatin oxide began on gestation day six. In rats, implantation is generally regarded as occurring five to six days after conception (e.g. Miller 1983). The U.S. EPA guidelines for developmental toxicity risk assessment (U.S. EPA 1991) make the following statement regarding preimplantation losses:

> "If treatment begins around the time of implantation (i.e. day 6 of gestation in the mouse, rat or rabbit), an increase in preimplantation loss probably reflects variability that is not treatment-related in the animals being used, but the data should be examined carefully to determine if there is a dose-response relationship. If preimplantation loss is related to dose, further studies would be necessary to determine the mechanism and extent of such effects."

Two developmental studies were conducted in Dutch rabbits (Shell 1973a). The second study was conducted due to difficulty in interpreting the results from the first study. In the first study, Study A, there was an increase in resorptions plus early fetal deaths per litter at the low dose (3 mg/kg/d) but not the high dose (10 mg/kg/d). Statistical significance values for the observations were not reported by the authors. Maternal deaths occurred in the vehicle control and both treatment groups in a non dose-related fashion. In Study B, the number of resorptions plus early fetal deaths per litter was increased in a dose-related manner. Measures of statistical significance for this observation were not reported. As in the earlier study, maternal deaths occurred in the negative control and both treatment groups in a manner apparently unrelated to treatment.

In a New Zealand White rabbit developmental study (Shell 1981), there were dose-dependent increases in mean post-implantation losses at the mid and high doses (5 and 10 mg/kg/d). However, pairwise comparisons between control and treated groups were not

statistically significant using the Wilcoxon Rank sum test; trend tests were not reported. There was a high (60%) and statistically significant percentage of abortions at the high dose. Maternal deaths were observed in control (2/18; 11%), mid dose (2/18, 11%) and high dose (5/23; 22%) groups, but not in the low dose or thalidomide positive control groups.

The increase in post-implantation losses at the mid dose in this study is one of the effects referred to in the citation supporting addition of fenbutatin oxide to the TRI list (US EPA 1994a).

The U.S. EPA developmental toxicity guidelines (US EPA 1991) make the following statement regarding maternal toxicity:

> "Agents that produce developmental toxicity at a dose that is not toxic to the maternal animal are especially of concern because the developing organism is affected but toxicity is not apparent in the adult. However, the more common situation is when adverse developmental effects are produced only at doses that cause minimal maternal toxicity: in these cases, developmental effects are still considered to represent developmental toxicity and should not be discounted as being secondary to maternal toxicity. At doses causing excessive maternal toxicity (that is, significantly greater than the minimal toxic dose), information on developmental effects may be difficult to interpret and of limited value."

In a section on study design, minimal toxicity is described as follows:

> "The high dose is selected to produce some minimal maternal or adult toxicity (i.e. a level that at the least produced marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity, and at the most produces no more than 10% mortality)."

In the developmental study in New Zealand White rabbits (Shell 1981), maternal mortality at the high dose (22%) exceeded the level in the controls (11%) by 11%. While the difference is not statistically significant ($p=0.3$), its biological significance is unclear.

In one of two multigeneration rat studies (Hine Laboratories 1973), litter size in the high concentration (300 ppm) group was consistently smaller than controls, although this was statistically significant only for the F1b litter. Parental body weights were reduced at this high concentration. In the second study (du Pont 1990), there was no consistent or statistically significant effect on litter size at concentrations of fenbutatin oxide at up to 500 ppm. Parental body weights were also reduced at the high concentration in this study. In the rat developmental study, no effect on litter size was found (Shell 1980b). It should be noted that comparison of the developmental and the reproductive studies is complicated by the use of a different strain of rats in each study. In the earlier rat reproduction study (Hine Laboratories 1973), reduced postnatal survival was observed in both litters of the third generation. Reduced pup weight at weaning was consistently found. In the later rat reproduction study (du Pont 1990), no effect on pup survival was found. It should be noted that this was a two-generation study, so does not directly contradict the finding of reduced pup survival in the third generation of the earlier study. Pup body weights were also reduced during lactation in the later study.

The statistically significant reduction in postnatal survival in the three-generation reproductive study was one of the effects referred to in the citation supporting addition of fenbutatin oxide to the TRI list (US EPA 1994a).

As the statute is currently interpreted, developmental effects resulting from postnatal exposure are outside the purview of Proposition 65 (OEHHA 1996). It is possible that the effects on lactating pups which were seen in the rat reproductive studies were due to adverse effects which occurred prenatally, but were manifested postnatally. It is also possible that they were due to transfer of fenbutatin oxide through the milk, or from pups eating their mother's food. Additionally, it is possible that they were due to problems with lactation in the mother. Hence, in the absence of data obtained from a study protocol involving cross-fostering of pups, the observed postnatal effects cannot be conclusively attributed to prenatal exposure to fenbutatin oxide.

F.2 Female Reproductive Toxicity

Data on female reproductive toxicity of fenbutatin oxide come from two multigeneration reproductive studies in rats, supplemented with acute studies in rats and chronic studies in rats, mice, and dogs. In both of the reproductive studies both sexes were treated. No human studies were located.

A three-generation reproductive toxicity was conducted in rats with two litters per generation using three concentrations of fenbutatin oxide in the food (Hine Labortories1973: section D.2.1). A small but consistent reduction in litter size was observed at the high concentration of fenbutatin oxide. This reduction was statistically significant only for one litter out of six (the F1b litter). Parental weights were also reduced at the high concentration.

A subsequent two generation rat reproduction study used four concentrations of fenbutatin oxide in food (du Pont 1990: section D.2.2). No effect on litter size was found. Parental weights were reduced at the high concentration. This later study used larger numbers of animals and was reported in much greater detail than the earlier study.

The three generation study found statistically significantly reduced pup survival during lactation at the high concentration in the third generation only. The two generation study found no effect on pup survival during lactation. Pup body weights were reduced in both studies during lactation.

Gross and/or histopathological examinations found no adverse effects on ovaries or uteri attributable to fenbutatin oxide treatment in three acute studies in rats, or chronic studies in rats, mice, or dogs (IBTL 1972a, 1972b, 1972c, Shell 1973a, 1973b, 1973c, du Pont 1989a, US EPA 1994: section D.3).

F.3 Male Reproductive Toxicity

Data on male reproductive toxicity of fenbutatin oxide come from two multigeneration reproductive studies in rats, a dominant lethal study in mice, acute and subchronic studies in rats, and chronic studies in rats, mice, and dogs. In both of the reproductive studies both sexes were treated. No human studies were located.

A three-generation reproductive toxicity was conducted in rats with two litters per generation using three concentrations of fenbutatin oxide in the food (Hine Labortories1973: section E.2.1). A small but consistent reduction in litter size was observed at the high concentration of fenbutatin oxide. This reduction was statistically significant only for one litter out of six (the F1b litter). Parental weights were reduced at the high concentration.

A subsequent two generation rat reproduction study used four concentrations of fenbutatin oxide in food (du Pont 1990: section E.2.2). No effect on litter size was found. Parental weights were reduced at the high concentration. This later study used larger numbers of animals and was reported in much greater detail than the earlier study.

No evidence for dominant lethal or other reproductive effects was found in a dominant lethal study in mice. However, it is not clear if a systemically toxic dose was used (Shell 1972: section E.3).

There is some evidence of effects of fenbutatin oxide treatment on testes weight in rats. In a one month study, both relative and absolute testes weights were increased in the presence of reduced body weights (SRI 1970: section E.4.2). In a two year study, both relative and absolute testes weights were increased at term. However, absolute testes weight was not increased at 3, 6, or 12 months, and less than half of the animals survived to term (Shell 1973b: section E.4.3).

In the offspring of treated rats, effects in the opposite direction were found. In a three-generation study, the relative testes weights of F3b weanlings was reduced. Body weight of the weanlings was also reduced (Hine Laboratories 1973: section E.2.1). In a two-generation study, the absolute testes weights were statistically significantly reduced in the mature F1 animals. However, the relative weights were increased. Body weights were also reduced (du Pont 1990). A two year study in dogs found no effect on testes weights.

Gross and/or histopathological examinations found no adverse effects on testes attributable to fenbutatin oxide treatment in three acute studies in rats, a subchronic study in rats, or chronic studies in rats, mice, or dogs.

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